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Ecophysiology and population genetics of Frangula caroliniana (Walt) Gray (Rhamnaceae)

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NOTE TO USERS

Ecophysiology and population genetics of *Frangula caroliniana* (Walt.) Gray (Rhamnaceae)

by

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This is to certify that the doctoral dissertation of

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has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

To Aimee and our girls (Goose, Choopa, and Emi-bemi)

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ABSTRACT

Frangula caroliniana (Walt.) Gray (or *Rhamnus caroliniana* Walt.) (Carolina buckthorn) is a woody species distributed throughout the southeastern United States. Due to its ornamental characteristics and the various soil conditions in which it grows, there is interest in promoting the species as a nursery crop within and beyond its native range. Concerns about the invasive potential of *F. caroliniana* need to be alleviated before it is promoted in horticultural commerce. These concerns are based on the aggressive spread of related species introduced to North America, including *Rhamnus cathartica* L. (common buckthorn). Thus, I sought to assess the landscape fitness of *F. caroliniana* by comparing some of its ecophysiological traits to those of *R. cathartica*. In addition, I determined the genetic structure of *F. caroliniana* through analysis of populations indigenous to 16 states. *Frangula caroliniana* fixed carbon at rates that permitted its survival in soils that ranged from dry to wet, but plants with inundated roots did not survive. Cold stratification at 4 °C for up to 112 days enhanced seed germination of *F. caroliniana*, but its seeds were more resistant to germination than were seeds of *R. cathartica*. Vernal bud break of *F. caroliniana* occurred 5.7 days later than that of *R. cathartica*, and depth of cold hardiness of *F. caroliniana* (-21 °C) may permit use of provenance-based selections of the species in regions where winters are harsher than those of the native habitat. While fruit set per unit stem length and unit leaf area of *F. caroliniana* was only 41% of that of *R. cathartica*, seedlings of both species established similarly in field soils. Analysis of amplified polymorphism fragment length (AFLP) markers revealed two distinct groups of genotypes of *F. caroliniana*; the first group was comprised of plants from South Carolina, which had the highest source of genetic variation, and the second consisted of the other sampled *F. caroliniana* populations

from 15 states. I conclude that the fitness for managed landscapes of *F. caroliniana* is promising, and that *F. caroliniana* lacks the capacity to be as invasive as *R. cathartica*.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Over the past several hundred years, most floral invasions in natural areas in North America have been due to plants that were introduced for horticultural use by nurseries, botanical gardens, and individuals (Reichard, 1997; Reichard and White, 2001). Woody invasive plants in particular were introduced for horticultural purposes. For example, Thomas Jefferson may have initially introduced the widely invasive *Cytisus scoparius* (L.) Link (Scotch broom) into the United States for ornamental purposes (Wyman, 1969). *Lonicera japonica* Thunb., a native of China, was introduced into the United States in the early 1800s and has invaded many parts of the country (Dirr, 1998; Zheng et al. 2004).

Many invasive woody species that were introduced for their ornamental appeal have common traits that make them appealing for use in managed landscapes, which are prone to periodic episodes of environmental stress (Kjelgren et al., 2000). For example, *Rhamnus cathartica* L. (common buckthorn) was initially introduced in the northwestern United States in the 1800s because of its cold hardiness, low susceptibility to herbivory and pathogens (Billington, 1949; Gourley, 1985), high tolerance to water stress (Stewart and Graves, 2004), and the ease of managing it as a hedge plant (Gourley, 1985). After a lag time of several decades (Gourley, 1985; Kowarik, 1995), the species escaped cultivation and has aggressively spread throughout the United States and Canada (Archibold et al., 1997). Some traits that make *R. cathartica* a successful invader include its high fecundity (Archibold et al. 1997; Kollmann and Grubb 1999), tolerance of varying degrees of water stress (Stewart and Graves, 2004), and the unique phenological and physiological characteristics of its foliage (Harrington et al., 1989). Nearly 29% of the annual carbon gain of *R. cathartica* in a

Wisconsin forest occurred before leaf emergence of a native shrub, *Cornus racemosa* Lam. (Harrington et al., 1989). *Rhamnus cathartica* also fixed carbon at a higher rate than did *C. racemosa* (Harrington et al., 1989). Not only is it a successful invader, but *R. cathartica* is also an alternate host of crown oat rust (*Puccinia coronata* Corda) (Leonard, 2003) and soybean aphid (*Aphis glycines* Mats.) (Voegtlin et al., 2004).

There are several species of *Rhamnus* L. s.l. that are native or naturalized in North America (Grubov, 1949), but few of them have the combined benefits of ornamental appeal and landscape fitness necessary for introduction into managed areas throughout the United States. There are also concerns about the invasiveness of other *Rhamnus* s.l. spp., but most problems in North America have resulted from only *R. cathartica* and another closely related invasive species, *Frangula alnus* Mill. (or *Rhamnus frangula* L.) (glossy buckthorn) (Catling and Porebski, 1994).

Frangula caroliniana (Walt.) Gray (or *Rhamnus caroliniana* Walt.) is an attractive shrub or small tree that is widely distributed across the southeastern United States. It is found as far north as southern Indiana and as far south as central Florida and southwestern Texas (Little, 1977). In its natural range, *F. caroliniana* occurs in valleys along streams and in highly acid sands (Simpson, 1999), on wooded rocky slopes, upland ridges, and commonly on limestone glades (Brizicky, 1964). The species, which can grow to about 12 m tall (Dirr, 1998), has glossy green leaves that turn orange, yellow, and red in the fall, and forms an open crown of slender branches. The fruits of *F. caroliniana* progressively change from green to light yellow to red, and finally turn blueberry-blue to black in late autumn (Graves, 2002). Similar to other members of Rhamnaceae, the flowers of *F. caroliniana* are small and inconspicuous (Brizicky, 1964).

Frangula caroliniana appears to exhibit a high degree of stress tolerance in the wild, suggesting that it could be promoted for use in horticultural landscapes. Concerns about its invasive potential and its apparent lack of sufficient cold hardiness in the Upper Midwestern United States, however, need to be resolved. Additionally, if *F. caroliniana* is shown to not be invasive, information concerning its genetic variation is necessary for cultivar selection and to understand the overall biology of the species. To reach this end the goals of my dissertation work were to:

1. determine how root-zone water content affects gas exchange and growth of *F. caroliniana* and to contrast these responses to those of *R. cathartica*;
2. characterize effects of cold stratification on seed germination of *F. caroliniana*;
3. assess the level of fecundity in mature branches of field-grown plants of *F. caroliniana* and *R. cathartica*;
4. compare the phenology of vernal bud break of *F. caroliniana* and *R. cathartica*;
5. determine the depth of cold hardiness of *F. caroliniana* and *R. cathartica* inside and outside the native range of *F. caroliniana*;
6. describe patterns of growth, carbon fixation, and carbon allocation over a growing season of seedlings of *F. caroliniana* and *R. cathartica*; and
7. assess the geographical pattern of genetic variation in wild populations of *F. caroliniana*.

A powerful and effective way of predicting the invasiveness of a species is by comparing its ecophysiological performance with ecologically and taxonomically similar invasive taxa (Mack, 1996; Pattison et al., 1998; Morris et al., 2002; Daehler, 2003). A comparative approach has been considered useful in understanding what make species

invasive (Rejmánek, 1995; Mack, 1996; Grotkopp et al., 2002) and was the basis for my rationale for including *R. cathartica* in all but one (i.e., population genetics study) of the experiments.

Dissertation Organization

This dissertation consists of five manuscripts. The first manuscript, chapter 2, has been published in *HortScience* and is formatted for that journal. The second manuscript, chapter 3, has been published in *HortScience* and is formatted for that journal. The third manuscript, chapter 4, is formatted for submission to the *Journal of the American Society for Horticultural Science*. The fourth manuscript, chapter 5, is formatted for submission to *Oecologia*. The fifth manuscript, chapter 6, is formatted for submission to the *American Journal of Botany*. Also included is a literature review and general conclusions synthesized from the studies that comprise this dissertation.

Literature Review

Water stress

Water is one of the most limiting resources for plant life (Kramer and Boyer, 1995), and plants vary in their response to high or low water availability (Baker, 1974; Cleverly et al., 1997). Due to the extremes in water availability of managed landscapes, land managers need plants that are able to tolerate periodic episodes of drought and flooding. Woody plants in managed landscapes often experience extremes in soil moisture availability due to disturbed and poor rhizosphere conditions (Harris et al., 1999). Factors contributing to the unsatisfactory soil environment include compaction, low soil nutrient levels, and extremes in edaphic temperatures (Berrang et al., 1985; Graves et al., 1989; Kielbaso, 1990). Invasive plants, however, readily take advantage of these disturbed environments (Baker, 1974;

Bazzaz, 1986), because of their high tolerances to environmental heterogeneity (Sakai et al., 2002) and their capacity to exploit generally high resource levels in these areas (Davis et al., 2000). Invasive species have higher resource-use efficiencies than do native species (Baruch and Goldstein, 1999), which includes the efficient use of water during drought and flooding.

During drought, *Acer platanoides* L., an invasive species in the eastern United States, utilized water more efficiently than did a native congener, *Acer saccharum* Marsh. (Kloeppel and Abrams, 1995). The invasive *Ruellia tweediana* Griseb. exhibited greater photosynthetic rates under dry or wet conditions than did the native *Ruellia caroliniensis* Steud. (Wilson et al., 2004). A widely distributed invasive wetland species, *Phragmites australis* (Cav.) Trin. ex Steud., was tolerant of varying levels of standing water (Coops et al., 1996). *Phalaris arundinacea* L. was productive under a wide range of moisture levels, including flooding and drought (Miller and Zedler, 2003). An increase in water supply increased the invasibility of plant communities in dry regions, either by directly increasing the water supply or by improving access to mineral nutrients (Davis et al., 1999; Davis et al., 2000). Indeed, high water-use efficiency was an important trait for populations of *Impatiens capensis* Meerb. during adaptation to dry conditions (Heschel et al., 2002). However, in a survey of physiological and morphological traits of invasive and native plant species in a tallgrass prairie community, invasive species as a group did not differ from native species in resource-use efficiency (Smith and Knapp, 2001). Moreover, in a review of 79 comparisons between native and invasive plants, Daehler (2003) found only 10 comparisons that showed consistent advantages of the invader over the native species. The most common growing conditions that favored native species over successful invaders were environments with low-resource availability. Daehler (2003) concluded that increased resource availability and altered

disturbance regimes increase the performance of invasive species over that of native species. Consequently, rather than just comparing the performance of taxonomically and/or ecologically similar invasive and non-invasive species under high-resource conditions, more comparative work needs to be done to assess the performance of these invaders under resource-poor conditions, where native plants generally outperform invasive plants (Davis et al., 2000; Sher et al., 2000; Smith and Knapp, 2001; Daehler, 2003).

Reproductive biology

Seed germination

There is little, if any, information on the reproductive biology of *F. caroliniana*. There are limited data available on *F. alnus* (Medan, 1994; Hampe and Bairlein, 2000; Bolmgren, 2004; Hampe, 2005), but how much of this information can be extrapolated to *F. caroliniana* is unknown because the taxa belong to different sections of the *Frangula* genus (*F. alnus*: *Eufrangula* Grub.; *F. caroliniana*: *Cascara* Grub.) (Grubov, 1949), and the taxonomy of the genus remains to be thoroughly examined (Medan, 1994).

Frangula caroliniana is characterized by small, inconspicuous, 5-merous, hermaphroditic flowers (Grubov, 1949; Johnston and Johnston, 1978) that are typically pollinated by insects (Brizicky, 1964; Medan and Schirarend, 2004). And like other members of Rhamnaceae, the taxon has petal-opposed stamens (Richardson et al., 2000; Medan and Schirarend, 2004). The fleshy, drupaceous fruits of *F. caroliniana* are 3-ocular (Grubov, 1949).

Although *F. caroliniana* is easily propagated via vegetative means (Graves, 2002), information about its sexual reproductive characteristics is needed for those who wish to propagate it for horticultural purposes and for others who want to better understand the

relationship of the species' fecundity with its invasive potential. Although seeds of some members of Rhamnaceae exhibit physical dormancy (Turner et al., 2005), it appears that most *Frangula* species exhibit endodormancy (Youngblood, 2003), which is typically overcome by cold (≈4 °C), moist stratification (Bewley and Black, 1994).

Fecundity

High fecundity is a commonly held trait of successful invaders (Baker, 1974; Bazzaz, 1986; Sakai et al., 2001), including *Spartina alterniflora* Loisel. (Callaway and Josselyn, 1992), *Banksia ericifolia* L. f. (Honig et al., 1992), *Gleditsia triacanthos* L. (Marco and Páez, 2000), *Sapium sebiferum* L. (Siemann and Rogers, 2001), and *Eschscholzia californica* Cham. (Leger and Rice, 2003). Invasive species typically have continuous seed production, high fruit loads, and a lack of special requirements for germination (Baker, 1974).

The high fecundity of *R. cathartica* is well known (Archibold et al. 1997; Kollmann and Grubb 1999), but scant information is available regarding the fecundity of species in the *Frangula* or *Rhamnus* s.s. genera. The number of potential offspring of a fragmented *F. alnus* population in southern Spain was estimated to be 430 to 1560 individuals per year (Medan, 1994). Fewer than 1% of flowers of *Rhamnus alaternus* L. produced seedlings that survived beyond 1 year (Gulias et al., 2004). On average, fewer than 50 ripe fruits developed of nearly 150 flowers of *F. alnus* trees in southern Spain (Hampe, 2005). Yields of *Frangula californica* (Eschsch.) Gray and *Frangula purshiana* (DC.) Cooper are 11 seeds/g and six seeds/g, respectively (Piper, 1986). There is a clear need for more information on the reproductive biology of *F. caroliniana* and the *Frangula* genus in general.

Ecophysiology

Vegetative phenology

The timing of growth and reproductive activity, phenology, varies among species (Gurevitch et al., 2002) and climates (Olsson and Ågren, 2002), but is considered to be a major determinant of the range of woody species (Chuine and Beaubein, 2001). Some exotic woody species are invasive due to their unique phenological characteristics relative to native flora (Kloeppel and Abrams, 1985; Harrington et al., 1989; Schierenbeck, 1992). The early bud break of *R. cathartica* has been mentioned, but *Lonicera japonica* Thunb. also exhibits earlier patterns of bud break relative to native species (Schierenbeck, 1992). Buds expanded, however, at about the same time for the invasive *Ligustrum sinense* Lour. and the native *Forestiera ligustrina* (Michx.) Poir, which are both in Oleaceae (Morris et al., 2002). Within its native range in Sweden, the North American invader, *Lythrum salicaria* L., initiated growth earlier in more northern populations (Olsson and Ågren, 2002).

Cold hardiness

Climate, specifically low temperature, is one of the most limiting factors of the natural distribution of woody plants (Parker, 1963; Woodward, 1987; Lindén, 2002). The capacity of plants to withstand sub-freezing temperatures without sustaining long-term damage is an important criterion for evaluating the horticultural potential of a species (Lindén, 2002). The depth of cold hardiness of a species dictates where it can be appropriately planted (Harris et al., 1999). The cold hardiness of some species is greater than that required by the climate of their natural range (Parker, 1963; Flint, 1972; Pellett, 1981; Schrader and Graves, 2003; Sharma and Graves, 2004), which has both horticultural and ecological implications.

Although long-term field studies can provide accurate assessments of the depth of cold hardiness (Flint, 1972; Alexander et al., 1984), quicker methods are needed for the estimation of cold hardiness of woody plant species (Lindstrom and Dirr, 1991; Lindén, 2002). Various methods of assessing the depth of cold hardiness of woody species have been in use for several years (Stergios and Howell, 1973; Lindén, 2002; Schrader, 2002). These methods, typically done in laboratory settings with freezers, include electrolyte leakage testing (Dexter et al., 1930; Flint et al., 1967; Whitlow et al., 1992), tetrazolium staining (Malone and Ashworth, 1991; Sharma and Graves, 2004), differential thermal analysis (Quamme, 1991), and visual observation (Stergios and Howell, 1973; Pellett et al., 1981; Schrader and Graves, 2003). I decided to use the visual observation method to assess the depth of cold hardiness of *F. caroliniana* and *R. cathartica* due to its simplicity and accuracy.

Growth and carbon fixation

Invasive species sometimes have traits that allow them to dominate native plant communities (Allison and Vitousek, 2004). These traits include high specific leaf area (leaf area per unit leaf dry mass) (Reich et al., 1997; Baruch and Goldstein, 1999; Allison and Vitousek, 2004), rapid growth (i.e., relative growth rate) (Pattison et al., 1998; Stratton and Goldstein, 2001), and high photosynthetic capacity (Lambers and Poorter, 1992; Baruch and Goldstein, 1999; Stratton and Goldstein, 2001).

Specific leaf area is important component of plant growth and development because it leads to a high proportion of leaf area per unit dry mass invested and high photosynthetic capacity (Reich et al., 1997). It is also one of the main determinants of relative growth rate (Poorter and de Jong, 1999). While there is a strong correlation between high specific leaf area and high relative growth rate for many species of similar life forms (Lambers and

Poorter, 1992; Lambers et al., 1998; Reich et al., 1997; Baruch and Goldstein, 1999), it does not always explain the effectiveness of invasive species outcompeting native species (Smith and Knapp, 2001; McDowell, 2002). Instead, net assimilation rate (plant dry weight per unit leaf area per unit time) may be the driving factor associated with relative growth rate (Lambers et al., 1998) and is considered to be a functional trait of some successful invaders (Pattison et al., 1998).

Interspecific differences exist in the relative growth rate of plants (Poorter, 1990), but the high relative growth rate of some invasive species is attributable to their high photosynthetic rates (Pattison et al., 1998). Relative growth rate is also a key indicator of the performance of species in their natural habitats (Cornelissen et al., 1996).

Population genetics

Frangula caroliniana is a member of the Rhamnaceae, which is estimated to be anywhere from 62 (Wikström et al., 2001) to 96 (Basinger and Dilcher, 1984; Richardson et al., 2000) million years old. Rhamnaceae is a cosmopolitan family with nearly 50 genera and 900 species that consist of trees, shrubs, climbers, and one herb (Richardson et al., 2000). Recent work utilizing plastid DNA sequences has placed Rhamnaceae within the order Rosales along with other families such as Elaeagnaceae, Barbeyaceae, Dirachmaceae, Urticaceae, Ulmaceae, Moraceae, and Rosaceae (Chase et al., 1993; Judd et al., 1994).

Globally, the *Rhamnus* s.l. genus is comprised of 125 (Johnston and Johnston, 1978) to 142 (Grubov, 1949) species that are mainly found temperate and neotropical areas of the Northern hemisphere. However, some species are found in South America and even southern Africa (Grubov, 1949; Johnston and Johnston, 1978). The name of the genus,

Rhamnus, was used by Theophrastus (300 B.C.) to describe a low, thorny thicket (probably *Rhamnus oleoides* L.) in the Greek archipelago (Grubov, 1949).

Grubov (1949) recognized 52 species of *Frangula* that are found throughout the world, but the highest concentration of species (24) is located in Central and Southern America. The *Frangula* genus is not universally recognized (de Candolle, 1825; Weberbauer, 1895; Suessenguth, 1953; Brizicky, 1964). Although Johnston and Johnston (1978) conceded that Frangula is a monophyletic group in its own right, they still considered the genus closely related to *Rhamnus* s.s. and emphasized that "hierarchic rank at which these relationships are to be recognized is not a scientific question but one depending on tradition, usage, and the practical consideration of the optimization of communication." Regardless, recent molecular phylogenetic data (Richardson et al., 2000; Bolmgren and Oxelman, 2004) have confirmed the distinct separation of *Frangula* from *Rhamnus* s.s., and has also been found to have diverged prior to *Rhamnus* s.s. (Richardson et al., 2000). Several morphological characters of *Frangula* also justify the taxonomic separation of the two genera, which include its deciduous habit; 5-merous hermaphroditic flowers; naked winter buds; and nearly straight, pinnate leaf nervation (Grubov, 1949; Kartesz and Gandhi, 1994). At least within the United States, *F. caroliniana* and *Rhamnus caroliniana* Walt. are synonymous. The argument of Johnston and Johnston (1978) is persuasive. Hence, I opted to identify *F. caroliniana* as *R. caroliniana* in three chapters of my dissertation that primarily serve a horticultural audience. In the remaining chapters I identify the taxon as *F. caroliniana*.

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CHAPTER 2. PHOTOSYNTHESIS AND GROWTH OF *RHAMNUS CAROLINIANA* DURING DROUGHT AND FLOODING: COMPARISONS TO THE INVASIVE *RHAMNUS CATHARTICA*

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Abstract. Rhamnus caroliniana Walt. (carolina buckthorn or Indian cherry) is an attractive small tree or shrub found in diverse habitats in the United States. Because the species occurs in both mesic and xeric soils, we questioned whether selections of carolina buckthorn could be marketed as new nursery crops resistant to both drought and flooding. Our first objective was to characterize how soil water affects growth and gas exchange of carolina buckthorn. We studied potted plants subjected to soil moistures that ranged from complete submersion of the root zone to severe drought (7% soil water by volume). The maximal photosynthetic rate occurred at 27% soil water content, and complete submersion killed plants. Our second objective was to compare responses of carolina buckthorn to those of the invasive common buckthorn (*Rhamnus cathartica* L.) when potted plants were treated with partial flooding of root zones and drought. Carolina buckthorn resisted deleterious effects of partial flooding. In contrast, leaves of common buckthorn became epinastic, and rates of photosynthesis were low (2.14 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) after 17 days of treatment. Mean photosynthesis of common buckthorn increased to 5.52 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, a rate similar to that of carolina buckthorn,

after 55 days of treatment. Drought reduced net photosynthesis by 52 and 68%, respectively, for carolina buckthorn and common buckthorn relative to rates of plants in the control treatment. We conclude that carolina buckthorn is capable of maintaining carbon fixation and growth over a wide range of soil water contents, and unlike common buckthorn, is not dependent upon morphological, anatomical, or physiological adjustments to optimize growth and net photosynthesis in extremely wet soil. Use of carolina buckthorn as an ornamental is warranted if invasiveness and other potential problems with the species are not identified.

Carolina buckthorn is used rarely in horticultural landscapes but is a distinctive shrub or small tree native to the Lower Midwestern and southeastern United States. In its natural range, carolina buckthorn occurs on alluvial soils as well as on dry, upland, limestone ridges (Brown and Kirkman, 1990; Foote and Jones, 1989). The various soil conditions associated with indigenous carolina buckthorn imply that the species is adapted to diverse soil water contents typical of managed landscapes. However, the response of carolina buckthorn to extreme soil moisture has not been evaluated.

The resistance of carolina buckthorn to different soil water contents will help to define how the species might be used horticulturally. But, regardless of its resilience in poor soils, acceptance of carolina buckthorn as a new horticultural crop for landscaping also will depend on other factors, most notably its potential to be invasive. All species commonly called buckthorns (the entire *Rhamnus* L. genus) often are assumed to be invasive even though most problems in North America have resulted from only two species, *Rhamnus cathartica* L. (common buckthorn) (Archibold et al., 1997; Gourley, 1985) and *Rhamnus frangula* L. (glossy buckthorn) (Catling and Porebski, 1994). The introduction of these

species to North America from other continents has led to negative consequences for natural ecosystems in some regions. In a southern Wisconsin urban forest, Harrington et al. (1989) found that common buckthorn had greater midday photosynthetic rates (9 to 13 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) than a native woody species, *Cornus racemosa* Lam. In a forest near Ottawa, Ont., Berry et al. (1997) found that glossy buckthorn had a midday photosynthetic rate of 7 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, which was, on average, 97% greater than that of the other three understory species studied. Such observations, along with the lack of ornamental appeal of several *Rhamnus* spp., may explain why buckthorns rarely are grown or used by horticulturists.

We believe the outstanding growth habit, fruit display, and foliar traits of carolina buckthorn (Graves, 2001) make it an ornamentally superior member of its genus. Whether the species may become invasive has not been resolved, but knowing how resistant carolina buckthorn is to a range of soil water contents will be useful to those assessing the habitats in which the unintended escape and spread of the species might be possible. Thus, our rationale for studying how carolina buckthorn responds to soil-water conditions is two-fold. Our data will aid horticulturists by helping to define preferred soil conditions for carolina buckthorn used for ornamental landscaping and will aid ecologists who are assessing the invasiveness potential of the species. Our specific objectives were to determine how root-zone water content affects gas exchange and growth of carolina buckthorn and to contrast these responses to those of common buckthorn. The widespread region of North America where common buckthorn is naturalized includes Missouri, Illinois, Indiana, and Ohio, states where carolina buckthorn is indigenous. Our rationale for comparing these two species included their partially sympatric occurrence, the capacity of both to persist in diverse habitats (Brown

and Kirkman, 1990; Foote and Jones, 1989; Soper and Heimburger, 1982), and the opportunity to contrast carolina buckthorn to a closely related species known to be invasive.

Materials and Methods

Effects of soil water content on gas exchange and growth of carolina buckthorn. Thirty full-sibling, four-month-old carolina buckthorns indigenous to Johnston County, Okla. (lat. 34°19'40"N; long. 96°42'20"W) were grown in a greenhouse. Leaf area of five randomly chosen seedlings was determined on 1 Aug. 2000 with a leaf area meter (model 3100; LI-COR, Lincoln, Nebr.), and roots, stems, and leaves were separated, washed, dried in an oven at 67 °C for 3 d, and weighed. These data were used to assess treatment effects on growth of the remaining seedlings, which were cultured in 3.44-L pots (height = 16 cm, top diameter = 20.3 cm) filled with 5 *Sphagnum* peat : 3 perlite : 2 silt loam soil (by volume). Each pot was placed in a 6.19-L pot (height = 21.6 cm, top diameter = 21.6 cm) to facilitate flooding the root zone of some plants while sustaining similar root-zone temperatures for all plants. Five treatments (severe drought, moderate drought, moist, wet, and flooded) assigned randomly to 25 plants (n = five) were imposed in a greenhouse from 1 Aug. through 18 Sept. 2000. We irrigated to the water-holding capacity of drained root zones every 12, 8, and 4 d for plants in the severe-drought, moderate-drought, and moist treatments, respectively. The lower portion of the root zone of plants in the wet treatment was saturated continuously within a 2.5-cm-high saucer filled with tap water. Entire root zones in the flooded treatment were immersed in tap water by sealing the drainage holes in the 6.19-L pots and keeping the water column at the surface of the medium in the 3.44-L pots.

All plants were fertilized on 25 Aug. with 11.0-mM N from a mixture of Peters Excel All-Purpose and Cal-Mag (16.5N-2.2P-13.5K) (Scotts, Marysville, Ohio) in tap water. This

was the first day when plants in the moist, moderate-drought, and severe-drought treatments were scheduled for irrigation on the same date. Root zones in the wet and flooded treatments were flushed with fertilizer solution after briefly removing the 3.44-L pots from the 6.19-L pots. Two 400-W, high-pressure sodium lamps provided supplementary irradiance (90 $\mu mol \cdot m^{-2} \cdot s^{-1}$) between 0600 and 2200 HR. We used a data logger (model CR23X; Campbell Scientific, Logan, Utah) equipped with a model CS500 probe (Campbell Scientific) and a quantum sensor (model LI190SB; LI-COR) to determine mean air temperature (25.7 °C), mean relative humidity (56.6%), and mean photosynthetically active radiation during photoperiods (215 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Net photosynthesis of the youngest fully expanded leaf of the longest stem of each plant was measured with a model LI-6400 photosynthesis system (LI-COR) before irrigating on 25 Aug. and on 18 Sept. (the final day of treatment). Water content by volume of the upper 6 cm of the root zones was determined simultaneously with a ThetaProbe (model ML1; Delta-T Devices, Cambridge, UK). Leaf area and dry mass of roots, stems, and leaves were determined after roots were cleaned on 18 Sept. All tissues were oven-dried for 3 d at 67 °C. We also determined relative growth and net assimilation rates (Harper, 1977). All data were collected in a random order without regard to treatment.

Data were analyzed by using the general linear models procedure and Tukey's honestly significant difference option of SAS/STAT software, Version 8.2 (1999-2001) (SAS Inst., Cary, N.C.). We used regression analysis to determine the soil water content at which net photosynthesis was maximal.

Comparison of carolina buckthorn and common buckthorn. Seeds of carolina buckthorn were collected from one plant in Cook Station, Mo. (lat. 37°48'46"N; long.

91°26'16"W), one plant in Brazito, Mo. (lat. 38°26'44"N; long. 92°18'9"W), and two plants in Adams Co., Ohio (lat. 38°40'25"N; long. 83°27'10"W). Seeds also were collected from the plant from Oklahoma that was the seed source for the first experiment. The seeds were stratified in the autumn of 2000 and germinated in a greenhouse. Experimental units were first-year seedlings from each of the five maternal parents. The three provenances provided intraspecific variation. Seeds of common buckthorn from five plants naturalized in Ames, Iowa (lat. 42°2'5"N; long. 93°37'11"W) were stratified, and seedlings were produced, by using the same procedures and schedule used for carolina buckthorn. All plants were grown in 1.64-L pots (height = 15.2 cm, top diameter = 15.2 cm) containing Fafard mix #3-BF (Fafard, Anderson, S.C.). Thirty plants (15 per species, three per sibling group) were randomly assigned to each of three treatments (control, drought, and partial flood). The 90 plants were treated from 1 Aug. through 16 Oct. 2001.

We monitored water content of root zones with the sensor used during the first experiment. Control plants were irrigated when mean soil water content of root zones for both species decreased to 27%, which was the soil water content at which maximal net photosynthesis occurred in the first experiment. Plants in the partial-flood treatment were held in 18-cm-diameter saucers that contained a 7.2-cm column of tap water that entered root zones via drainage holes in the bottom of the pots. When mean water content of root zones in the drought treatment decreased to 10%, we fertilized all plants with a solution of the same fertilizer and concentration used during the first experiment. This was done on 1, 17, and 31 Aug.; 12 and 25 Sept.; and 5 and 16 Oct., dates that defined the end of repeated drought cycles. Before fertilization on each day a drought cycle ended, net photosynthesis and root-zone water were measured as during the first experiment. Supplementary irradiance was

provided by four 400-W, high-pressure sodium lamps (116 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The environment was monitored as during the first experiment. Air averaged 25.5 °C, mean relative humidity was 60.1%, and mean photosynthetically active radiation during photoperiods was 344 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The procedures used during the first experiment were followed as we harvested plants destructively on 16 Oct.

We analyzed data by using the general linear models procedure and Tukey's honestly significant difference option (SAS Inst.). Regression analysis was performed to test for effects of duration of partial flooding on net photosynthesis of both species. Data from carolina buckthorn also were analyzed separately from those of common buckthorn to assess for differences between provenances.

Results

Effects of soil water content on gas exchange and growth of carolina buckthorn. All flooded plants died, most within the first 25 d of treatment, and all plants in the other four treatments survived. Leaves of the flooded plants began to wilt 15 d after treatment, which was followed by necrosis of the leaves and stems. Although the drought and wet treatments inhibited growth relative to the moist treatment, plants in all of these treatments appeared healthy and sustained glossy, green leaves.

Plants in the moderate-drought, moist, and wet treatments had similar mean photosynthetic rates (Table 1). Mean photosynthesis of plants in the moist treatment was more than double that of plants in the wet treatment, but there was considerable variation among plants. Regression analysis showed that the maximal rate of mean photosynthesis occurred when root zones contained 27% water by volume ($y = -0.35 + 23.9[water] -$

44.3[water^2], $r^2 = 0.44$). Root dry weight of plants in the wet treatment was 31% of that of plants in the moist treatment, whereas the flooded plants that died had no surviving roots (Table 1). Stem and leaf dry weights of moist-treated plants were greater than those of plants in the other treatments (Table 1). Plants in the wet and severe-drought treatments had lower total dry weights than did plants in the moist treatment. Plants subjected to moderate drought showed relative growth and net assimilation rates similar to those of plants in the moist treatment (Table 1).

Comparison of carolina buckthorn and common buckthorn. Net photosynthetic rate of partially flooded carolina buckthorn did not change over time; there was no evidence of a linear ($P = 0.96$) nor quadratic ($P = 0.28$) response (Fig. 1). However, the net photosynthetic rate of partially flooded common buckthorn was initially 59% less than that of carolina buckthorn but attained similar values by day 55 of treatment (Fig. 1). Common buckthorn initially showed leaf epinasty upon exposure to partial flooding but recovered as indicated by resumption of normal shoot development of new leaves distal to the epinastic leaves.

At the species level, the mean net photosynthetic rate of carolina buckthorn was 26% greater than that of common buckthorn ($P \leq 0.001$) (Table 2). Root ($P = 0.03$) and stem ($P \leq 0.001$) dry weights of common buckthorn were greater than those of carolina buckthorn (Table 2). Total plant dry weight of common buckthorn also was greater than that of carolina buckthorn ($P = 0.001$) (Table 2). The ratio of root to leaf dry weight of common buckthorn was 31% greater than that of carolina buckthorn ($P \leq 0.001$) (Table 2).

At the treatment level, mean net photosynthetic rates of the two species did not differ within irrigation treatments. The actual root-zone water content among plants in the drought treatment averaged 7% when photosynthesis was measured and root zones were rehydrated.

This led to reductions in photosynthesis of 52 and 68%, respectively for carolina buckthorn and common buckthorn relative to rates of plants in the control treatment (Table 2). Total leaf area and root, stem, and total plant dry weights of control-treated common buckthorn were greater than those of common buckthorn subjected to drought and partial flooding and those of all carolina buckthorns. The drought and partial-flood treatments had similar effects on carolina buckthorn except that net photosynthesis of partially flooded carolina buckthorn was greater than that of drought-treated carolina buckthorn (Table 2). Root-to-leaf ratio of common buckthorn was greater across treatments and within the drought treatment than that of carolina buckthorn (Table 2). There were no differences in average net photosynthetic rate ($P = 0.13$) and total plant dry weight ($P = 0.90$) among the three provenances of carolina buckthorn.

Discussion

Carolina buckthorn and common buckthorn fix carbon at rates that permit their survival in dry and moderately wet soils. However, common buckthorn appears to use carbon more efficiently than does carolina buckthorn, and common buckthorn undergoes adjustments in root zones that are extremely wet to optimize its growth and net photosynthesis. Such adjustments appear unnecessary for carolina buckthorns native to Missouri, Ohio, and Oklahoma to persist in soils with high water content. Thus, we conclude that regardless of provenance, carolina buckthorn is adapted to a wide range of soil-water conditions and is highly tolerant of both moderate degrees of drought and of root zones that are partially inundated.

Our approach to characterizing how water supply affects gas exchange and growth of carolina buckthorn was to make comparisons to common buckthorn, an aggressive invader of

natural landscapes in the United States. Common buckthorn, like several other *Rhamnus* spp. (Archibold et al., 1997; Catling and Porebski, 1994), prefers moist soil. Among species-treatment combinations, total plant dry weight and leaf area were greatest for common buckthorns treated as controls (Table 2). Although the reduced growth of common buckthorns we treated with drought might seem contradictory to previous claims that the species grows well at dry sites (Gourley, 1985; USDA, 1948), the drought effects we observed should be considered within the context of the overall vigor and resilience of this species. Drought did reduce growth, but the weight and leaf area of drought-stressed common buckthorns were not different from those traits of carolina buckthorns that were provided uniform water in the control treatment (Table 2). Moreover, carolina buckthorn showed no differences in total dry weight and leaf area after being exposed to both drought and partial flooding during our second experiment, an indication that carolina buckthorn will withstand wet and dry soils in managed landscapes. Similar results have been reported for *Acer rubrum* L. (Abrams and Kubiske, 1990) and *Nothofagus solandri* (Hook. f.) Oerst. (Sun et al., 1995), which, like carolina buckthorn, are indigenous to dry and wet climates. The relatively small impact of drought compared to wetness and flooding during our first experiment (Table 1) indicates that carolina buckthorn is particularly adapted to dry soils. Hence, unless subsequent research shows that other factors such as invasiveness preclude its use, carolina buckthorn should be considered by producers and consumers as a large shrub or small tree adapted to both dry and wet soils. Sites where soils become completely inundated should be avoided, however.

Relationships between photosynthetic rate and accrual of dry weight illustrate the importance of the extensive leaf surface area of common buckthorn and suggest that common

buckthorn uses resources more efficiently than does carolina buckthorn. Although at the species level carolina buckthorn had a photosynthetic rate that was 26% greater than that of common buckthorn, the comparatively low dry-matter accumulation of carolina buckthorn suggests that a relatively high portion of its fixed carbon was used for maintenance respiration. Accumulation of dry matter under soil-water conditions in our control treatment was 56% greater for common buckthorn than for carolina buckthorn, but mean photosynthetic rates for those plants did not differ (Table 2). The 31% greater total leaf surface area of control-treated common buckthorns compared to carolina buckthorns (Table 2) may explain this finding, and common buckthorn also may have a comparatively high resource-use efficiency. Invasive species typically use resources more efficiently (Baruch and Goldstein, 1999; Bazzaz, 1986) and have higher rates of CO_2 assimilation (Kloeppel and Abrams, 1995; Stratton and Goldstein, 2001) than do other species. The low assimilation rates at the species level for common buckthorn compared to carolina buckthorn that we found when measures were made at discrete times might be offset over a season in the field because leaves of common buckthorn emerge sooner and senesce later than those of carolina buckthorn (J.R. Stewart, unpublished data). In southern Wisconsin, 29% of annual carbon gain of common buckthorn occurred before leaf emergence of *Cornus racemosa* Lam. (Harrington et al., 1989). The comparatively short seasonal time span during which they are foliated may represent a mechanism that restricts the capacity of carolina buckthorns to be as invasive as common buckthorns. Net photosynthesis of carolina buckthorn in the moist treatment of the first experiment (Table 1) was low relative to that of carolina buckthorn in the control treatment of our second experiment (Table 2). According to Larcher (2003), the average maximum values for net photosynthesis of deciduous trees under field conditions

ranges from 6-12 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. Our data from the second experiment approach this range, but all mean photosynthetic rates from the first experiment were less than 3 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. Although the explanation for these low means is uncertain, feedback inhibition due to root restriction within the pots may have been a factor. Such inhibition can cause a downward regulation of photosynthesis (Arp, 1991; Thomas and Strain, 1991). Also, the plants in the first experiment were grown in a soil-based medium that might have had a relatively high bulk density. This could have impaired photosynthesis (Handreck and Black, 2002) of carolina buckthorn in the moist treatment relative to that of carolina buckthorn in the control treatment of the second experiment, which were grown in a soil-less medium.

The adjustment in photosynthetic rate over time among partially flooded common buckthorns may have been due to hormonal responses within the plant (Bradford and Yang, 1980). Transport of 1-aminocyclopropane-1-carboxylic acid from flooded root zones to shoots may explain our observations of leaf epinasty of common buckthorn (Taiz and Zeiger, 1998). It is also possible that the resumption of normal leaf development coincided with the generation of adventitious roots in response to hypoxic conditions induced by waterlogging (Chen et al., 2002; Schmull and Thomas, 2000). carolina buckthorn evidently lacks the need for such adjustments and resists deleterious effects of wet and partially inundated soils. Most flooded plants show reduced net photosynthesis (Bradford, 1983; Pezeshki et al., 1996) and dry matter (Chen et al., 2002) as we observed for partially flooded common buckthorns (Fig. 1, Table 2). Mechanisms underlying the intriguing capacity of common buckthorn to recover from stresses during long exposure to partial root-zone inundation merit further investigation.

Although caution is needed when extending the results of experiments performed in a greenhouse to field-grown plants, several researchers using similar techniques have shown

meaningful interspecific and intraspecific differences that are consistent in the greenhouse and the field (Myers and Landsberg, 1989; Schrader and Graves, 2000). Our work has demonstrated comparatively high leaf surface area combined with high ratios of root to leaf weight among common buckthorns. In addition, our data on common buckthorn suggest it uses resources more efficiently than does carolina buckthorn. This broadens the base of knowledge concerning factors that might contribute to the invasiveness of common buckthorn. These include dispersal of large annual seed crops by birds (Godwin, 1936; Kollmann and Pirl, 1995), vigorous plant development (Archibold et al., 1997), and possibly allelopathy (Boudreau and Wilson, 1992). While these traits have led to detrimental consequences for natural ecosystems in which common buckthorn has invaded, not all members of the Rhamnaceae are similarly problematic. For example, previous research indicates that *Rhamnus californica* Eschsch. (Paine et al., 1992; Schuch and Burger, 1997), *Ceanothus griseus* (Trel.) McMinn (Paine et al., 1992), and *Ceanothus americanus* L. (Martin et al., 1991) are stress-resistant plants that are not invasive. Carolina buckthorn appears similarly less prone to invasiveness and merits further evaluation for use as an ornamental plant for managed landscapes.

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Table 1. Mean photosynthetic rate, leaf area, plant dry weight, root-to-shoot ratio, relative growth rate, and net assimilation rate of potted *Rhamnus caroliniana* Walt. (carolina buckthorn) plants assigned to five soil-water treatments that were based on irrigation frequency. Treatments were imposed from 1 Aug. 2000 until 18 Sept. 2000. There were five single-plant replicates per treatment.

	Dependent variable								
			Dry weight (g)						
Treatment	Photosynthetic rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	Leaf area (cm^2)	Root	Stem	Leaf	Total plant	Root-to-shoot ratio	Relative growth rate ($g \cdot kg^{-1} \cdot d^{-1}$)	Net assimilation rate ($g \cdot cm^{-2} \cdot d^{-1}$)
Drought									
Severe	0.5b[z]	1252b	4.4abc	7.5bc	7.5b	19.4b	0.3a	7.2bc	0.9b
Moderate	1.7ab	1332b	5.9ab	7.7b	8.1b	21.7ab	0.4a	9.2ab	1.2ab
Moist	2.9a	2235a	8.8a	10.5a	13.2a	32.5a	0.4a	17.1a	3.0a
Wet	1.4ab	930b	2.7bc	5.1c	5.4b	13.3b	0.3a	-0.5c	0.1b
Flooded	0.0b	0c	0.0c	0.0d	0.0c	0.0c	0.0b	0.0c	0.0c

[z]Treatment means within each column followed by the same letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

Table 2. Mean photosynthetic rate, leaf area, plant dry weight, and root-to-leaf ratio of potted *Rhamnus caroliniana* Walt. (carolina buckthorn) and *Rhamnus cathartica* L. (common buckthorn) plants assigned to three treatments that were based on soil water content. Treatments were imposed on 1 Aug. 2001 and ended on 16 Oct. 2001. There were 45 single-plant replicates per species and 15 single-plant replicates per treatment.

	Dependent variable						
			Dry weight (g)				
Species and treatment	Photosynthetic rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	Leaf area (cm^2)	Root	Stem	Leaf	Total plant	Root-to-leaf ratio
Species across treatments							
Rhamnus caroliniana	4.8A[z]	560A	1.8B	0.9B	2.7A	5.4B	0.7B
Rhamnus cathartica	3.8B	583A	2.2A	2.3A	2.4A	6.8A	0.9A
Control treatment							
Rhamnus caroliniana	5.9a[y]	652b	2.2b	1.0c	3.1a	6.4b	0.7b
Rhamnus cathartica	5.2a	854a	3.1a	3.3a	3.6a	10.0a	0.9ab
Drought treatment							
Rhamnus caroliniana	2.8b	548bc	1.8bc	0.9c	2.8ab	5.4b	0.7b
Rhamnus cathartica	1.7b	478bc	2.1bc	2.0b	2.2ab	6.3b	1.1a
Partial-flood treatment							

Rhamnus caroliniana	5.8a	479bc	1.4c	0.8c	2.1bc	4.3b	0.7b
Rhamnus cathartica	4.7a	418c	1.3c	1.4bc	1.5c	4.3b	0.9ab

[z]Species means within each column followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

[y]Treatment means within each column followed by the same lower-case letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

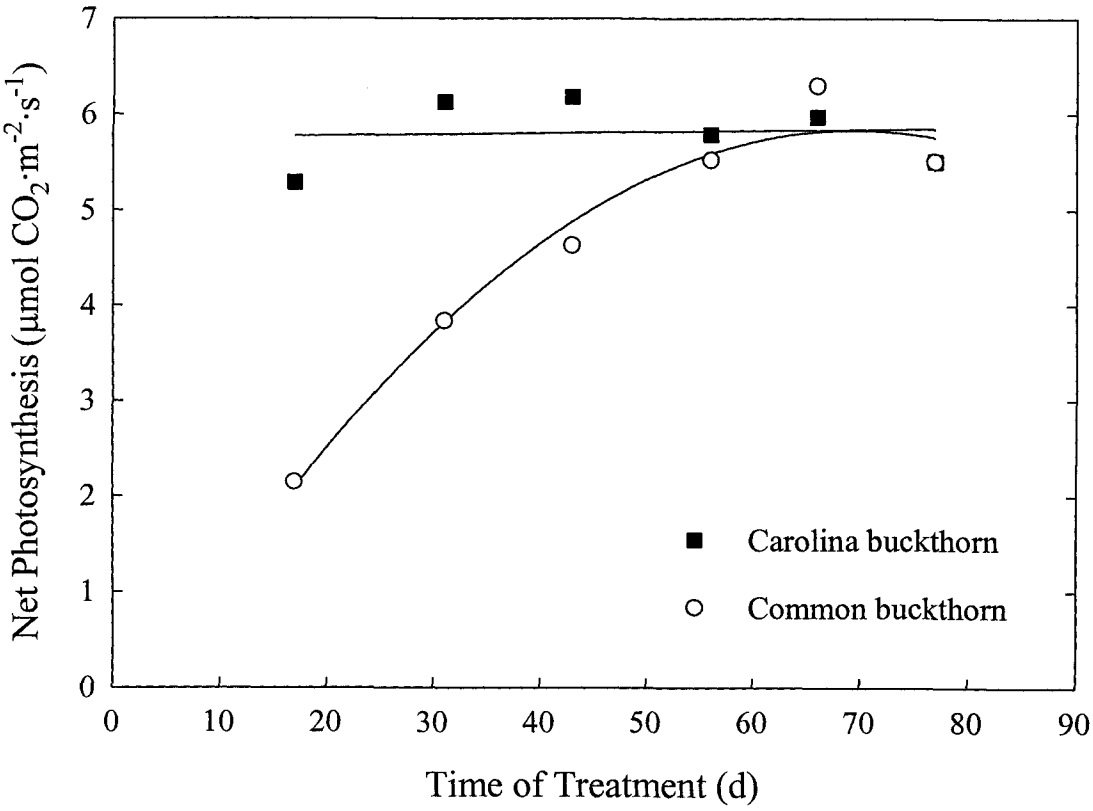

Fig. 1. Mean net photosynthesis of youngest, fully expanded leaves of partially flooded common buckthorn ($y = -0.7379 + 0.1892[day] - 0.0014[day^2]$, $r^2 = 0.97$) and partially flooded carolina buckthorn in the second experiment ($y = 5.749 + 0.0012[day]$, $r^2 = 0.01$). Values are means of 30 plants of each species.

CHAPTER 3. SEED GERMINATION OF *RHAMNUS CAROLINIANA*: IMPLICATIONS FOR ECOLOGY AND HORTICULTURE

A paper published in HortScience

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Additional index words. carolina buckthorn, indian cherry, *Frangula caroliniana*, common buckthorn, invasiveness, germination

Abstract. Little is known about the reproductive biology of carolina buckthorn [*Rhamnus caroliniana* Walt. or *Frangula caroliniana* (Walt.) Gray], an attractive North American shrub or small tree that might merit increased use in managed landscapes. The fecundity and high germinability of seeds of the Eurasian common buckthorn (*Rhamnus cathartica* L.), however, have been characterized as factors contributing to its invasiveness. We compared seed germination of these species to ascertain how easily carolina buckthorn could be grown from seed in nurseries and to acquire data for predicting whether carolina buckthorn might be invasive if introduced into managed landscapes. Fruits of carolina buckthorn were collected from indigenous plants in central Missouri, southern Oklahoma, and southern Texas. Fruits of common buckthorn were collected from shrubs naturalized in central Iowa. Seeds of both species were stratified for up to 112 days in darkness at 4 °C; germination at 24 °C in the dark was then evaluated for 56 days. Quadratic functions best described how time of stratification influenced germination value and germination percentage of common buckthorn, whereas these measures of carolina buckthorn were best represented by exponential (value) or linear (percentage) functions. Stratification for 112 days maximized germination value and

percentage for carolina buckthorn within the 56-d germination period, but shorter stratifications were sufficient to optimize germination of common buckthorn. While the overall mean germination of carolina buckthorn was 40%, results varied by provenance and ranged from 25% (Missouri) to 56% (Oklahoma). Mean germination of common buckthorn over times of stratification was 71%, and the overall mean daily germination of common buckthorn, 1.3, was 86% greater than that of carolina buckthorn, 0.7. We conclude that seeds of carolina buckthorn are more resistant to germination than seeds of common buckthorn. Our results suggest that plant propagators should cold-stratify seeds of carolina buckthorn for up to 112 days, and suggest that carolina buckthorn has a lower potential to be invasive than does common buckthorn.

Carolina buckthorn, also known as indian cherry, is an attractive shrub indigenous to the south-central and southeastern United States. The species has glossy leaves that turn orange, yellow, and red in the autumn. Plants grow to about 12 m tall (Dirr, 1998) and form open crowns of slender branches. The distinctive fruits of carolina buckthorn progressively change from green to light yellow to red, and finally turn blueberry-blue to black in late autumn (Graves, 2002). Across its natural range, the species is found in riparian areas as well as on dry, upland, limestone ridges (Brown and Kirkman, 1990; Foote and Jones, 1989). Consistent with this pattern of occurrence, young plants of carolina buckthorn tolerate both drought and partially flooded soils (Stewart and Graves, 2004).

Its stress resistance (Stewart and Graves, 2004), ease of vegetative propagation (Graves, 2002), and ornamental appeal suggest carolina buckthorn could be promoted for use in horticultural landscapes. Several members of the *Rhamnus* L. genus, including common

buckthorn (*Rhamnus cathartica* L.), however, are invasive in the north-central and northeastern United States. Although there are several reasons for the pervasiveness of common buckthorn in disturbed habitats (Gourley, 1985), the fecundity of common buckthorn is a dominant element contributing to its invasiveness (Archibold et al., 1997; Hubbard, 1974; Kollman and Grubb, 1999). Gourley (1985) cited the high viability and rapid germination of common buckthorn seeds as factors that contribute to the aggressive invasiveness of the species, and germination percentage of common buckthorn exceeded that of seeds from two cultivars of *Rhamnus frangula* L. (Wheeler and Starrett, 2001). The buried seed bank beneath mature plants of common buckthorn averaged 620 viable seeds·m^{-2} in natural areas near Saskatoon, Saskatchewan, and the mean germination rate of overwintered seeds of common buckthorn was 85% (Archibold et al., 1997). These findings are consistent with several reports indicating that introduced species that become invasive have higher rates of germination than those of closely related native species (Mihulka et al., 2003; Radford and Cousens, 2000; van Clef and Stiles, 2001).

Little information is available about the reproductive biology of carolina buckthorn. Fresh seeds of carolina buckthorn may require no pre-treatment to germinate (Esquivel, 2001; Nokes, 2001). However, fruits of the species do not ripen until late in the growing season and often are retained on plants through at least early winter. Thus, in a temperate climate, seedlings from these fruits are not likely to establish due to the onset of winter. It has been suggested that stored seeds should be stratified for 30 d at 5 °C to evoke germination within five weeks (Nokes, 2001), but no data related to seed germination of carolina buckthorn have been published.

Our objective was to characterize effects of cold stratification on seed germination of carolina buckthorn. Our rationale for this research was 2-fold. Because carolina buckthorn is an appealing, native species (Dirr, 1998) that may merit use in managed landscapes (Graves, 2002), our data should benefit those who may wish to propagate plants for commerce. In addition, our results provide new insights about the degree of resistance to germination inherent among seeds of carolina buckthorn. While far from describing the invasive potential of the species fully, this information represents a first step toward that goal, particularly because germination of seeds of carolina buckthorn from multiple provenances was compared with germination of the invasive common buckthorn. Including multiple seed sources in our experiments was important because of interspecific and intraspecific variation in resistance to germination of seeds of members of the *Rhamnus* L. genus (Hubbard, 1974; Young and Young, 1992).

Materials and Methods

Fruits of carolina buckthorn were collected during October 2001. Collections were made from five plants in Brazito, Mo. (lat. 38°26'44"N; long. 92°18'9"W), from five plants along the Blue River in Johnston County, Okla. (lat. 34°19'40"N; long. 96°42'20"W), and from five plants in Kerrville, Texas (lat. 30°02'50"N; long. 99°08'24"W). Fruits of common buckthorn were collected from five naturalized plants in Ames, Iowa (lat. 42°2'5"N; long. 93°37'11"W). Seeds were separated from fruits 7 to 14 d after collection and stored in paper bags at 20 °C for three months until treatment initiation. Only seeds weighing ≥ 20 mg each were used. Seed moisture content on 5 Apr. 2002 was 4.2% and 5.5% for carolina buckthorn and common buckthorn, respectively (International Seed Testing Association, 1985).

Each experimental unit consisted of 34 half-sibling seeds from one maternal source that were held between two pieces of 90-mm-diameter filter paper within a 100- × 15-mm plastic petri dish. Due to seed availability, there were 23 half-sibling seeds per petri dish from one of the five maternal sources in Missouri. Each experimental unit to be stratified received 1.75 mL distilled H_2O and then was placed into a dark growth chamber at 4 °C. Dates in 2002 when stratification began differed based on the various ascribed durations so that the date treatments ended was the same (7 May) for all experimental units. Treatment durations of 0, 14, 28, 42, 56, 84, and 112 d were initiated on 7 May, 23 Apr., 9 Apr., 26 Mar., 12 Mar., 12 Feb., and 15 Jan., respectively. Experimental units were arranged in a completely randomized design with five replications (each with seeds from only one of the five maternal sources) per treatment (common buckthorn) or provenance within treatment (carolina buckthorn). On May 7 we applied 3.5 mL distilled H_2O to experimental units assigned to the 0-d treatment, applied an additional 1.75 mL to all other units, and then randomized them all in a dark growth chamber at 24 °C. Each petri dish was sealed in an individual plastic bag to minimize moisture loss during the stratification and germination periods. During the germination period, we added 1 to 1.75 mL distilled H_2O to experimental units in which the filter paper began to appear dry (every 5 to 10 d). Filter paper was replaced as needed to prevent fungal growth.

Germination was defined as the emergence of a radicle, which was confirmed by viewing seeds with a 15× dissecting microscope. Germinated seeds were counted every 3 to 4 d for 56 d, and germination value (Czabator, 1962), germination percentage, germination distribution, peak day, peak value, and mean daily germination were calculated (Schrader and Graves, 2000a). Germination value is the product of peak value and mean daily

germination and is a composite expression of the speed and completeness of germination (Czabator, 1962), which is useful for measurement of seeds of woody plants that may germinate slowly. Germination percentage is the number of germinated seeds divided by the total number of seeds in a given seed lot. Mean daily germination is the total germination percentage divided by the number of days of the germination period. Germination distribution is the number of days over which germination occurred. The day during the germination period on which the highest number of seeds germinated is defined as the peak day. Peak value is the cumulative germination percentage for each experimental unit on its peak day, divided by the number of days that were required to reach that percentage. Tetrazolium tests (Peters, 2000) were performed after the final day of germination assessment to determine the viability of ungerminated seeds.

The effects of species and time of stratification on seed germination were analyzed by analysis of variance and Tukey's honestly significant difference option of SAS/STAT software, Version 8.2 (SAS Inst., Cary, N.C., 1999-2001) using the general linear models procedure. Data from carolina buckthorn also were analyzed separately from those of common buckthorn to assess for differences between populations. Analysis of variance showed no interaction between the main effects of species and time of stratification. There was also no interaction between the main effects of populations of carolina buckthorn and time of stratification (Table 1). We were able to analyze these effects separately due to their independence (Cochran and Cox, 1992). Regression analysis was performed to test for effects of cold stratification on seeds of both species.

Results

Averaged over all stratification periods, germination value did not differ between species as a whole, but germination percentage of common buckthorn was 78% greater than that of carolina buckthorn (Table 1). Tetrazolium tests revealed that 16% and 49% of the ungerminated seeds of common buckthorn and carolina buckthorn were viable, respectively. Germination distribution, peak day, and peak value of both species were similar (Table 1). Mean daily germination of common buckthorn was nearly twice that of carolina buckthorn (Table 1).

The germination value of seeds of carolina buckthorn from Oklahoma was three times that of seeds from Missouri (Table 1). Seeds from Oklahoma germinated at the highest percentage among the three provenances and showed a greater germination distribution than did seeds from Missouri (Table 1). There were no differences among provenances in peak day or peak value, but seeds from Oklahoma had the highest mean daily germination (Table 1).

Germination value of seeds of carolina buckthorn increased exponentially as time of stratification increased; values ranged from 0.2 at 0 d of stratification to 9.9 at 112 d of stratification (Fig. 1A). Germination value of seeds of common buckthorn changed quadratically over time of stratification and was as high as 4.7 at 56 d of stratification but decreased thereafter to 3.9 at 112 d (Fig. 1A). Germination percentage of seeds of carolina buckthorn increased linearly over time of stratification from 18% to 69% (Fig. 1B). Germination percentage of common buckthorn was 48% after 0 d of stratification, increased to 85% with 42 d of stratification, and declined slightly at 112 d (Fig. 1B). Time of stratification decreased peak day for seeds of carolina buckthorn linearly from 36 d at 0 d of

stratification to 9 d at 112 d (Fig. 1C). Peak day for non-stratified seeds of common buckthorn was similar to that of non-stratified carolina buckthorn, but the decrease in peak day over time of stratification for common buckthorn ceased after 42 d (Fig. 1C). Peak value of seeds of carolina buckthorn increased exponentially over time of stratification from 0.3 at 0 d of stratification to 7 at 112 d (Fig. 1D). At 0 d of stratification, peak value for common buckthorn was 1 (Fig. 1D). It increased to 3 at 56 d of stratification and decreased to 2.6 at 112 d (Fig. 1D). Mean daily germination of carolina buckthorn increased linearly over time of stratification from 0.3 to 1.2 %/d (Fig. 1E). Mean daily germination of common buckthorn consistently exceeded that of carolina buckthorn, and the linear increase due to stratification was less pronounced for common buckthorn than for carolina buckthorn (Fig. 1E). Quadratic regression functions best described the relationship of time of stratification and germination distribution of both species (*Rhamnus caroliniana*: $y = 14.2 + 0.38[day] - 0.0025[day^2]$, $r^2 = 0.87$; *Rhamnus cathartica*: $y = 18.4 + 0.32[day] - 0.0023[day^2]$, $r^2 = 0.78$). Germination distribution for seeds of carolina buckthorn was 17 d at 0 d of stratification, reached a maximum of 30 d at 56 d of stratification, and declined to 26 at 112 d. Similarly, germination distribution for seeds of common buckthorn was 19 d at 0 d of stratification, 32 d after 84 d, and only 24 d at 112 d.

Discussion

Our results are significant to horticulture in three ways. First, we have shown that cold stratification enhances the germination of seeds of carolina buckthorn. Second, the inclusion of seeds from different provenances of carolina buckthorn in our work indicates that the germination characteristics of the species vary with seed source. Lastly, we conclude that, in general, seeds of carolina buckthorn are more resistant to germination than are seeds

of common buckthorn. Although more research is needed to assess whether carolina buckthorn merits increased horticultural use, our findings provide potential growers with useful information on propagation and represent a first step toward resolving questions regarding the potential invasiveness of the species.

Both germination value and germination percentage increased with increasing duration of cold stratification for seeds of carolina buckthorn, but the pattern of trends over time differed. The exponential increase in germination value over time of stratification shows that relatively long stratification periods are particularly beneficial (Fig. 1A). In contrast, increases in germination percentage (Fig 1B) and mean daily germination (Fig. 1E) were linear, indicating that improvements in how quickly and uniformly seeds germinate, rather than gains in germination percentage, account for the more pronounced increases in germination value predicted by the exponential function after day 60 (Fig. 1A). This conclusion is consistent with other indicators of the speed of germination; as time of stratification increased, peak day decreased (Fig. 1C) and peak value (Fig. 1D), which is derived from peak day, increased. While these results illustrate the benefits of cold-stratifying seeds, the minimal duration of stratification considered acceptable will vary among growers of carolina buckthorn and will depend on the source of the seed (Table 1); on average, about 80 d should elicit 50% germination (Fig. 1B), and at least 112 d would optimize germination value (Fig. 1A). Future research should be designed to determine the dormancy mechanisms that account for the relatively high percentage of viable seeds of carolina buckthorn that do not germinate after cold stratification.

Research representing more provenances is needed to determine whether the degree of resistance to germination among seeds of carolina buckthorn is random or can be predicted

based on geographic origin. The provenance differences we observed are consistent with previous work with other woody species from temperate climates. For example, seeds of *Betula papyrifera* Marsh. from the northern limits of its natural distribution germinate faster than seeds of southern origin (Bevington, 1986), and germination varies among seeds of *Alnus maritima* (Marsh.) Nutt. depending on the disjunct provenance from which seeds are obtained (Schrader and Graves, 2000a). No cultivars of carolina buckthorn have been selected. If the low germinability of seeds from Missouri is consistent among plants within that provenance and over multiple years of seed development, selections from that region may have an inherently low likelihood of becoming invasive when used in amenity landscaping. The environmental benefits of low seed germination, however, must be reconciled with the need for higher seed germination by growers.

We included common buckthorn in this research so that the influence of cold stratification on seed germination of carolina buckthorn could be compared to that of a closely related species that is known to be invasive. The comparatively high germination percentage and mean daily germination of common buckthorn over times of stratification (Table 1) indicate that seeds of carolina buckthorn are comparatively resistant to germination, particularly when times of stratification are brief (Fig. 1B, E). While these data provide some basis to claim that carolina buckthorns introduced into horticultural landscapes may prove less invasive than common buckthorns, it is important to recognize the limitations over what can be concluded. While fecundity and high seed viability and germination lead to the invasiveness of some *Rhamnus* spp. introduced to North America from other continents (Archibold et al., 1997; Gourley, 1985; Hubbard, 1974; Kollman and Grubb, 1999; Wheeler and Starrett, 2001), our results alone do not permit definitive conclusions about the potential

for invasiveness of carolina buckthorn. Field studies to evaluate the reproductive biology of the species could be designed to determine seed viability, the fate of seeds consumed by animals, and environmental factors that influence the establishment of unintended seedlings. In particular, work needs to be done to determine annual fruit and seed production of carolina buckthorn to verify our postulation that plants from Missouri might be less invasive than other seed sources. Seeds of some woody plants are less tolerant of low temperatures than are the plants on which they arise (Schrader and Graves, 2000b). Although plants of carolina buckthorn may survive if introduced to areas with colder winters than those where the species is native, seeds that develop on plants installed in cold climates may lose viability during winter. Pooler et al. (2002) showed that seeds from an interspecific cross of an invasive species introduced to North America, *Celastrus orbiculatus* Thunb., and its North American relative, *Celastrus scandens* L., were held under weaker dormancy than were seeds from intraspecific crosses of *C. scandens*. Thus, whether carolina buckthorn can hybridize with invasive members of its genus also should be determined.

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Table 1. Measures of germination of seeds of *Rhamnus cathartica* (common buckthorn) and *Rhamnus caroliniana* (carolina buckthorn) from Missouri, Oklahoma, and Texas. Seeds were stratified for 0, 14, 28, 42, 56, 84, and 112 d in the dark at 4 °C. Data are the means over the seven stratification periods. Values for species are means of 35 and 105 multi-seed replications for *Rhamnus cathartica* and *Rhamnus caroliniana*, respectively. Values partitioned by provenance are means of 35 multi-seed replications.

	Germination					Mean daily
Species and population	Value	%	Distribution (d)	Peak day	Peak value	germination (%/d)
Rhamnus cathartica	3.5A[z]	71.2A	25.2A	25.8A	2.3A	1.3A
Rhamnus caroliniana	2.7A	39.9B	23.6A	25.1A	2.0A	0.7B
Missouri	1.3b[y]	25.2b	16.8b	25.0a	1.5a	0.4b
Oklahoma	4.0a	56.3a	28.9a	26.3a	2.6a	1.0a
Texas	2.6ab	38.3b	25.1ab	24.0a	2.1a	0.7b

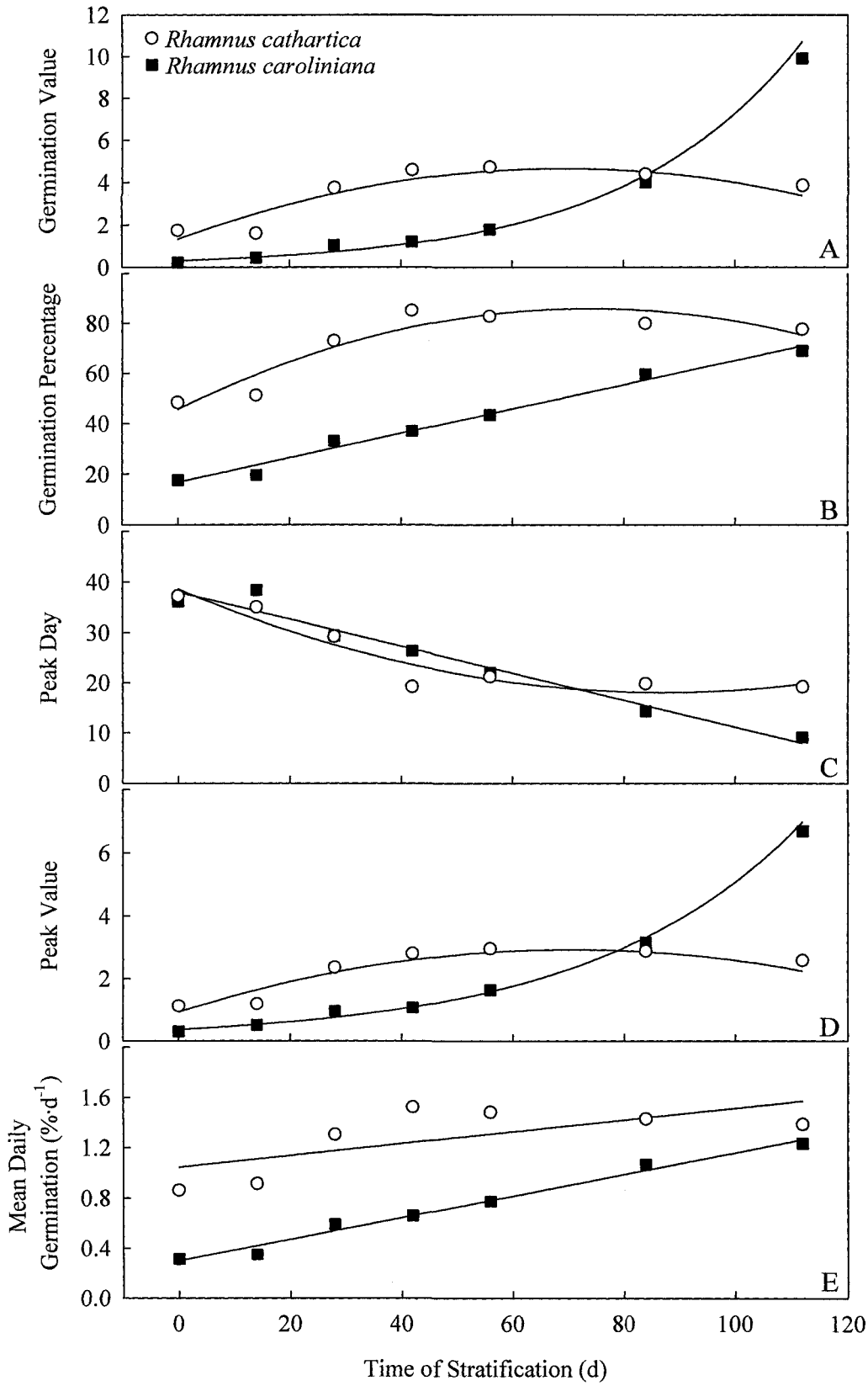
		Germination					Mean daily
Mean square	df	Value	%	Distribution (d)	Peak day	Peak value	germination (%/d)
Population (Pop)	2	63	8558***	1337**	45	12	3***

Time of stratification (Time)	6	177^{***x}	5556***	487	1748***	76	2
Pop × Time	12	23	193	281	95	6	0.1
Error	84	21	782	266	247	7	0.3

[z]Species means within each column followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

[y]Provenance means within each column followed by the same lower-case letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

[x]**,*** Significant at $P \leq 0.01$ or 0.001, respectively.


Rhamnus cathartica
Rhamnus caroliniana
Germination Value
Germination Percentage
Peak Day
Peak Value
Mean Daily Germination (%·d-1)
A
B
C
D
E
Time of Stratification (d)

CHAPTER 4. COLD HARDINESS AND VERNAL BUD BREAK OF *RHAMNUS CAROLINIANA* AND *RHAMNUS CATHARTICA*

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Additional index words. carolina buckthorn, *Frangula caroliniana*, common buckthorn, cold hardiness, phenology

Abstract. Carolina buckthorn (*Rhamnus caroliniana* Walt. or *Frangula caroliniana* [Walt.] Gray) is an attractive and stress-resistant shrub or small tree distributed extensively in the southeastern United States. Due to substantial climatic differences within its distribution (30-year normal midwinter minima range from 13 to -8 °C), selection among provenances based on differences in cold hardiness is warranted. Before selections are marketed, the potential of carolina buckthorn to be invasive also merits attention. Ecological problems resulting from the introduction of *Rhamnus* L. spp. in the United States, most notably the dominance of *Rhamnus cathartica* L. (common buckthorn) over neighboring taxa, are due in part to early bud break. Consequently, we investigated depth of cold hardiness and vernal bud break of carolina buckthorn and common buckthorn. To characterize cold hardiness, stem samples were collected on 5 Oct. 2002 and 11 Jan. and 2 Apr. 2003 from a field plot in Ames, Iowa in which common buckthorns of locally naturalized genotypes and carolina buckthorns native to Missouri, Ohio, and Texas had been established. Stem samples also were collected from common buckthorns and from carolina buckthorns native to Missouri

and Texas established in replicate plots in Ames, Iowa and New Franklin, Mo. on 16 Oct. 2003 and 14 Jan. and 12 Apr. 2004. Stem samples of carolina buckthorn and common buckthorn survived midwinter temperatures as low as -21 °C and -24 °C, respectively. Although the cold hardiness of carolina buckthorns from Missouri was greater than that of carolina buckthorns from Ohio and Texas on 2 Apr. 2003, there were no differences in cold hardiness of carolina buckthorns from Missouri and Texas on all three assessment dates in the second experiment. All plants survived at both field locations except for the carolina buckthorns from southern Texas planted in Iowa, which showed 0 and 17% survival in 2003 and 2004, respectively. Bud break of both species with and without mulch in Ames, Iowa was recorded from 9 Apr. to 10 May 2002. Mean bud break of common buckthorn was 5.7 days earlier than bud break of carolina buckthorn, and buds of mulched carolina buckthorns broke 4.2 days earlier than did buds of unmulched carolina buckthorns. We conclude that the cold hardiness of carolina buckthorn is sufficient to permit the species to be planted outside of its natural distribution. Populations of carolina buckthorn in Ohio and Missouri should be the focus of efforts to select genotypes for use in regions with harsh winters. Phenology of its bud break suggests carolina buckthorn will not be as invasive as common buckthorn, but evaluation of additional determinants of invasiveness is warranted.

Rhamnus caroliniana (carolina buckthorn or indian cherry) is a widely distributed, attractive shrub or small tree indigenous across the southeastern United States. This open-crowned species averages 12 m in height at maturity and has glossy leaves with distinctive autumn color (Dirr, 1998; Graves, 2002). Recent interest in promoting carolina buckthorn as a nursery crop is due to its ornamental features, its resistance to drought and flooding

(Stewart and Graves, 2004), and the ease with which it can be propagated vegetatively (Graves, 2002) and sexually (Stewart and Graves, 2005). Due to its extensive natural distribution, it is possible that genotypes of carolina buckthorn that are unusually cold hardy could be selected for use where winters are more harsh than those where the species is native. Within the northern portion of its native range in central Missouri, carolina buckthorn is exposed to 30-year normal midwinter minima ranging from -6 to -8 °C (National Climatic Data Center, 2005). In contrast, the species also occurs in central Florida, where the normal midwinter minima range from 11 to 13 °C (National Climatic Data Center, 2005).

Although winter temperatures influence the distribution of many woody species (Firbas, 1949), the cold hardiness of some species is greater than that required by the climate of their natural range (Flint, 1972; Parker, 1963). *Taxodium distichum* (L.) L.C. Rich. has a southerly native distribution in the United States, but has been grown as far north as Syracuse, NY (Dirr, 1998). *Alnus maritima* (Marsh.) Muhl. ex Nutt. has overwintered in Chanhassen and Lake George, Minn., where 30-year normal midwinter minima are -16 to -20 °C (National Climatic Data Center, 2005), even though it naturally occurs only in south-central Oklahoma, northwestern Georgia, and on the Delmarva Peninsula, where the 30-year normal mimina are 0 to -3 °C (National Climatic Data Center, 2005). Survival of *A. maritima* at -80 °C also has been demonstrated (Schrader and Graves, 2003). Intraspecific variation in cold hardiness correlates with latitude for some temperate woody species (Flint, 1972). For example, *Fraxinus americana* L. from northern provenances was more cold hardy than were conspecific plants from southern provenances (Alexander et al., 1984). Researchers studying *Quercus rubra* L. (Flint, 1972) and *Pinus strobus* L. (Lu et al., 2003) have shown similar

trends. Selections of carolina buckthorn could be suitable for horticultural use in regions with harsh winters if their cold hardiness is sufficient.

In conjunction with assessing the cold hardiness of carolina buckthorn, concerns about its potential to be invasive need to be addressed. Two members of the *Rhamnus* L. genus, *Rhamnus cathartica* (common buckthorn) and *Rhamnus frangula* L. (glossy buckthorn), were introduced into the northeastern United States and the maritime provinces of Canada in the 1800s (Gourley, 1985; Wyman, 1971). After a lag time of several decades, they escaped cultivation, naturalized, and have aggressively spread throughout the northern United States (Catling and Porebski, 1994; Gourley, 1985).

One reason common buckthorn invades natural areas is its relatively high seasonal carbon gain that is a consequence of early vernal bud break and foliation relative to neighboring deciduous species in the understory. Mean duration of foliation of common buckthorn exceeds that of native shrubs by up to 58 d (Barnes, 1972). Harrington et al. (1989) found that 29% of the annual carbon gain of common buckthorn occurred before leaf emergence of the native *Cornus racemosa* Lam. in Wisconsin. Early leaf emergence of common buckthorn was deemed more important to invasiveness than was late foliar senescence (Harrington et al., 1989). In comparison to a native congener, *Lonicera sempervirens* L., Schierenbeck and Marshall (1993) found that a long period of foliation contributed to the invasiveness of *Lonicera japonica* Thunb. Similar conclusions were made by Farnsworth and Meyerson (2003) concerning invasions of *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha angustifolia* L. in wetlands.

Congeneric pairs often are studied to predict traits responsible for invasiveness (Gerlach and Rice, 2003; Mack, 1985; Schierenbeck and Marshall, 1993). Because its

invasiveness in regions of the United States with harsh winters is well documented, common buckthorn is an ideal congeneric model species for ecological assessments of carolina buckthorn. Our objectives were two-fold. First, we determined the depth of cold hardiness of plants of carolina buckthorn from different provenances in comparison to that of common buckthorn. Second, we compared the timing of bud break of the two buckthorn species planted in the Upper Midwestern United States. Our data will benefit horticulturists seeking information on potential new nursery crops and will aid ecologists interested in the traits associated with the invasiveness of certain *Rhamnus* spp.

Materials and Methods

Cold hardiness. Common buckthorns and carolina buckthorns grown from seed from three provenances and established in a plot near Ames, Iowa, were used in the first of two experiments during the fall, winter, and spring of 2002-03. Samples of stems developed in 2002 were collected on 5 Oct. 2002 and 11 Jan. and 2 Apr. 2003 from three 2-year-old, half-sibling groups of common buckthorn and from 2-year-old, half-sibling groups of carolina buckthorn from three provenances: Cook Station, Mo. (lat. 37°48'46"N; long. 91°26'16"W), Gray Summit, Mo. (lat. 38°29'33"N; long. 90°49'0"W), Adams Co., Ohio (lat. 38°40'25"N; long. 83°27'10"W), and Kerrville, Texas (lat. 30°02'50"N; long. 99°08'24"W). We considered the two locations in Missouri the same provenance because they are within 160 km of each other (Hartmann et al., 1990). The common buckthorns were from naturalized populations in Ames, Iowa (lat. 42°2'5"N; long. 93°37'11"W). Each half-sibling group was four plants with the same maternal parent. We used a data logger (model CR10X; Campbell Scientific, Logan, Utah) equipped with a probe (model CS500; Campbell Scientific) to determine daily minimum temperatures at the research site. Thirty-year-normal daily minima

for the research and collection sites were obtained from the National Climatic Data Center (2005).

Sample twigs were put on ice and prepared within 48 h of collection. We cut each stem section to 4 cm in length after removing the terminal 2 cm of the distal end. Each section then was wrapped in moistened filter paper and inserted into a 16-mm-diameter culture tube that was held at 4 °C in a programmable freezer (ScienTemp, Adrian, Mich.) until the initiation of temperature ramping. We monitored freezer temperature by using a data logger (model CR23X; Campbell Scientific) and six type-T thermocouple wires arranged throughout the freezer. After all stem sections were prepared, the freezer temperature was lowered to -2 °C over 15 min. and held at -2 °C for 14 h to promote ice nucleation. Control samples then were removed and placed in a refrigerator at 4 °C, and then the freezer temperature was lowered at 2 °C per hour. Four tube-enclosed stem sections per source plant were removed at intervals of 4 °C. Removed samples were thawed on ice for 1 h and held in a refrigerator at 4 °C for 12 h. Thawed tubes were covered with parafilm and incubated in a dark growth chamber at 22 °C for 14 d. After incubation, each sample was cut longitudinally and deemed alive (if green) or dead (if brown) based on discoloration of the cambium and phloem. The lowest survival temperature (LST) of each plant source was considered as the minimum temperature at which three of the four stem sections were alive. The average of the four replicates per plant source was considered an experimental unit. Survival at five temperatures was evaluated on 7 Oct. 2002 (-4 to -20 °C) and 13 Jan. (-8 to -24 °C) and 2 Apr. 2003 (-8 to -24 °C). Survival of plants was also determined on 2 Apr. by assessing the hydration and integrity of stems, with particular attention to whether cambial tissue was tan to light green or had softened and become dark brown to black.

The second experiment occurred during the fall, winter, and spring of 2003-04. Plants established in field plots near Ames, Iowa, and New Franklin, Mo, were analyzed. Twigs formed in 2003 were collected on 16 Oct. 2003 and 14 Jan. and 12 Apr. 2004 from three 1-year-old, half-sibling groups of common buckthorn and from three 1-year-old, half-sibling groups of carolina buckthorn from two provenances. Plants were grown from seeds of carolina buckthorn collected from three plants in Brazito, Mo. (lat. 38°26'44"N; long. 92°18'9"W) and from three plants in Kerrville, Texas. Samples also were collected from three 2-year-old, half-sibling groups of common buckthorn grown from seed collected from naturalized plants in Ames, Iowa. Each half-sibling group consisted of ten plants with the same maternal parent. Daily temperature minima were monitored at the Ames plot as during the first experiment and at the New Franklin plot with a thermometer (Max/Min Temperature System, Airflo Instrument Co., Glastonbury, Conn.). Thirty-year normal minima for the research sites in Ames, New Franklin, and the collection sites were obtained from the National Climatic Data Center (2005). Fifty-year record daily minima for the research sites were obtained from the Climatology Index Page (The Weather Channel, 1995-2005).

Procedures used during the first experiment were followed, beginning within 12 h of collection. Survival of stem sections at five temperatures was evaluated on 21 Oct. 2003 (-4 to -20 °C) and 16 Jan. (-8 to -24 °C) and 13 Apr. 2004 (-8 to -24 °C).

Two linear models, one for species effects and one for provenance effects, were fit to the LST data of the first experiment. Linear models also were fit to the LST data of the second experiment. One model consisted of effects of species, location, and their interaction. The other model consisted of effects of provenance, location, and their interaction. Analyses were conducted with the PROC MIXED procedure in SAS/STAT® version 8.2.

Bud break. On 2 Oct. 2001, 128 carolina buckthorns and 32 common buckthorns, all 1-year-old seedlings, were planted in 16 adjacent plots near Ames, Iowa. Each plot was 1 m by 3 m, and each plant was an experimental unit in a completely randomized split-plot design. Each plot consisted of two common buckthorns (seeds from plants naturalized in Ames, Iowa), one carolina buckthorn from Brazito, Mo., six carolina buckthorns from Adams Co., Ohio, and one carolina buckthorn from Kerrville, Texas. Mulch treatments (mulch and no mulch) were randomly assigned to the whole plots, while plant species and provenance were assigned to the split plots. Fifteen cm of leaf (*Q. rubra*) mulch was placed on the soil surface of each plot assigned to the mulch treatment. Hardware cloth (hole diameter = 2.5 cm) was installed around each plot to stabilize the mulch.

We used a data logger (model CR23X; Campbell Scientific) connected to eight model 107 probes (Campbell Scientific) (four per treatment) and a model CS500 probe (Campbell Scientific) to determine temperature of soil (depth = 10.2 cm) of the mulched and non-mulched plots, and air, respectively, from 3 Nov. 2001 to 30 Apr. 2002 (Table 3). We monitored bud break of the plants every 1 to 3 d from 9 Apr. to 10 May 2002. First day of bud break (FDBB, the last day at which zero buds had broken), bud break percentage, and rate of bud break (RATE) were determined. For plants with broken buds on the first day of observation (8 out of 160), the first day of observation was considered to be FDBB. Bud break percentage was the number of buds broken on 10 May divided by the total number of buds (BTOT) on the plant. RATE was defined as:

$$RATE = BB*100/[(BTOT-BB_0)*(32-FDBB)] \quad \text{(Eq. 1)}$$

where BB_0 and BB are the number of buds broken on 9 Apr. and the number of buds broken each subsequent day, respectively.

We fit FDBB, bud-break percentage, and RATE to a linear-mixed model consisting of fixed effects of mulch, plants, the interaction of mulch and plants, and random effects of plots. The error associated with plots was used to test the main effect of mulch. We used contrasts of effects of plants and the interaction of mulch and plants to construct tests of the main effects of provenance and species. These effects also were used to construct tests of the interactions of mulch with provenance and mulch with species. Plants that did not survive (7 of 160) the winter were not included in the analyses, which were conducted with the PROC MIXED procedure in SAS/STAT® version 8.2. We found that there was an interaction between species and mulch for FDBB, bud-break percentage, and RATE for all three factors. We examined the effect of species at each mulch treatment for each dependent variable. The denominator degrees of freedom were calculated with the Kenward-Roger adjustment (Kenward and Rogers, 1997).

Results

Cold hardiness. Carolina buckthorn and common buckthorn were sufficiently cold hardy on all three assessment dates during the winter of 2002-03 relative to the 30-year normal minima for those dates (Table 1). Daily minima in late January and in early and late February were lower than the LST of carolina buckthorn on 11 Jan. (Fig. 1). Common buckthorn was more cold hardy than carolina buckthorn on 5 Oct., but there were no species-level differences on 11 Jan. and 2 Apr. (Table 1). Cold hardiness of carolina buckthorns from Missouri, Ohio, and Texas was similar on 5 Oct. and 11 Jan., while plants from Missouri were most hardy and plants from Texas were least hardy on 2 Apr. (Table 1). None of the carolina buckthorns from Texas survived to resume growth during spring 2003, but all

carolina buckthorns from Ohio and Missouri and all of the common buckthorn plants survived and resumed growth.

Carolina buckthorn and common buckthorn were adequately cold hardy in both Ames, Iowa and New Franklin, Mo. on all three assessment dates during the fall, winter, and spring of 2003-04 relative to the 30-year normal minima (Table 2). Daily minima in January and February were lower than the LST of carolina buckthorn on 11 Jan. for both locations (Fig. 2). Cold hardiness of common buckthorn was greater than that of carolina buckthorn on 16 Oct. in New Franklin, but not in Ames (Table 2). Cold hardiness of common buckthorn across locations was also greater than that of carolina buckthorn on 14 Jan. (Table 2). There were no differences in cold hardiness across locations between carolina buckthorns from Missouri and Texas on all three assessment dates, but only 17% of the plants from Texas planted in Ames, Iowa survived into April (Table 2). All plants from Missouri, however, survived and resumed growth during spring 2004.

Bud break. Mean minimum soil temperature in mulched plots over the winter and spring was 0.7 to 7.4 °C greater than that of unmulched plots (Table 3). The FDBB of common buckthorn (4.6 d after 9 Apr.) was not influenced by mulch and was earlier than that of carolina buckthorn (no mulch = 12.4 d after 9 Apr. [$P < 0.0001$], mulch = 8.2 d after 9 Apr. [$P = 0.02$]). Carolina buckthorns broke bud earlier when mulched than when not mulched (8.2 d after 9 Apr. vs. 12.4 d after 9 Apr. [$P = 0.005$]). There were no differences in the FDBB among carolina buckthorns from the three provenances ($P = 0.37$).

There was no mulch effect ($P = 0.75$) on the total percentage of broken buds of common buckthorn (92%), but 91% of buds broke on mulched carolina buckthorns, while only 61% of buds broke on plants without mulch ($P = 0.0004$). The bud break percentage of

common buckthorn (92%) was greater than that of carolina buckthorn (61%) in the unmulched treatment ($P < 0.0001$). There was no difference, however, in the total percentage of broken buds between mulched plants of the two species ($P = 0.59$). Only 38% of the unmulched carolina buckthorns from Texas survived, whereas 88% of the mulched plants from Texas survived. All carolina buckthorns from Missouri, regardless of treatment, and all unmulched plants from Ohio survived, while 98% of the mulched plants from Ohio survived.

Rate of bud break of common buckthorns was similar ($P = 0.99$), regardless of mulch (3.3 buds/d). Mulched carolina buckthorns (3.8 buds/d) had a greater ($P = 0.003$) RATE of bud break than carolina buckthorns in the no-mulch treatment (2.9 buds/d). There were no differences in RATE between carolina buckthorn and common buckthorn within the mulch ($P = 0.18$) and unmulched ($P = 0.08$) treatments.

Discussion

These data represent the first information on the potential cold hardiness of carolina buckthorn. Our approach was to plant seedlings from disparate provenances in central Missouri, near the northern limit of the geographical distribution for carolina buckthorn, and in central Iowa, ≈360 km to the northwest of the northern natural limit for the species. We designed our experiments to permit comparisons of carolina buckthorn and common buckthorn, an invasive Eurasian member of the same genus that is naturalized in both Missouri and Iowa. Common buckthorn is cold hardy throughout the Upper Midwest and thus served as a control to ensure the reliability of our methods.

Conclusions regarding cold hardiness can be drawn based on LST determinations made in October, January, and April in the laboratory and on survival in the field. If we

ignore differences among provenances of carolina buckthorn and make comparisons at the species level, the primary difference is that carolina buckthorn is less cold hardy than is common buckthorn during early and mid-October. Nonetheless, we also conclude that plants from all three provenances of carolina buckthorn we studied have the potential to survive winters harsher than those associated with their native habitats. Although further evaluation is needed, our data support the conclusion that the geographical area beyond the natural distribution in which cold hardiness will permit the safe use of carolina buckthorn varies with provenance of the germplasm.

Analyses showed the LST of carolina buckthorn averaged over provenances was 8 °C higher in October 2002 and 3 and 14 °C higher in Ames and New Franklin, respectively, in October 2003 than the LST of common buckthorn (Tables 1 and 2). Carolina buckthorn and common buckthorn showed similar LSTs at three of the four other measurement dates of both experiments, but the LST of carolina buckthorn was 7 °C higher than the LST of common buckthorn in mid-January of 2004 (Table 2). The higher LSTs of carolina buckthorn in October of both years may be due to genetically controlled patterns of cold acclimation related to the photoperiod found in the geographic range of carolina buckthorn. Increased cold hardiness of temperate woody plant species generally develops long before the lowest temperatures associated with the region occur (Kozlowski and Pallardy, 2002). Cold-hardening in woody plants is a two-stage process (Sakai and Larcher, 1987; Weiser, 1970). While the first stage is induced by an interplay of genetic control (Flint, 1972; Schrader and Graves, 2003), photoperiod, and temperature, the second stage is primarily initiated by temperatures < 0 °C (Weiser, 1970). Although the October LSTs of carolina buckthorn were higher than those of common buckthorn, there were no differences in LST of

the two species at other dates except for 14 Jan. 2004 (Tables 1 and 2). Genetic control of the second stage of cold-hardening in carolina buckthorn appears to be weaker than in the first stage and allowed the hardening process to occur in a manner similar to that of common buckthorn (Tables 1 and 2). Regardless, carolina buckthorn was sufficiently hardy in October to withstand the 50-year-record daily minima of both dates (Tables 1 and 2). Primary differences between the seasonal cold hardiness of both species may be related to provenance differences of sampled material of carolina buckthorn. Indeed, the Apr. 2003 survival rate of carolina buckthorns from Texas in 2004 was 0% (Table 1), and our decision to ascribe the hardiness of 0 °C to stem material from Texas, which was dead upon collection in April, likely resulted in a conservative estimate of its hardiness. There can be substantial variation in cold hardiness among provenances of woody plant species (Cannell and Sheppard, 1982; Schrader and Graves, 2003). The true cold hardiness of many woody plant taxa has often been underestimated and is often greater than assumed for many woody plant species with wide geographic ranges (Flint, 1972). Carolina buckthorn conforms to this pattern, but the difference between our estimates of its actual hardiness and the hardiness it would need to persist in the harshest portions of its native range is not as great as exists for some other taxa (Schrader and Graves, 2003).

Annual normal daily minima associated with the provenances of carolina buckthorn we used range from 0 °C (Kerrville, Texas) to -8 °C (Brazito, Mo.), and our observations over two years indicate that each provenance of carolina buckthorn we studied has the potential to persist when planted in regions in which normal daily minima are lower than those of the native habitat. For example, 100% of plants indigenous to southern Texas survived the winter of 2003-04 in central Missouri (Table 2), and all plants indigenous to

southern Ohio (Table 1) and central Missouri (Tables 1 and 2) survived in central Iowa. Although LST data show that carolina buckthorns from all provenances were sufficiently hardy at each sampling date to withstand the normal daily minima for Ames and New Franklin, our LST data suggest carolina buckthorns could not survive the 50-year-record minima for Ames in January (Tables 1 and 2) and for both locations in Apr. 2004 (Table 2). This illustrates the possibility that carolina buckthorns planted at sites where winter conditions are similar to those in Ames and New Franklin might be damaged or killed during unusually harsh winters. While this is an important issue that should be addressed, the fact that our data suggest that common buckthorns also develop inadequate hardiness relative to the record minima should be considered. The aggressive spread of common buckthorn in the Upper Midwest and into climatic zones in Canada represented by Winnipeg and Thunder Bay (Farrar, 1995) is evidence of hardiness greater than we determined. We may have underestimated the mid-winter hardiness of common buckthorn; its LST of -24 °C in January of both years was the lowest temperature to which we subjected stems. For the same reason, our LST estimates for carolina buckthorns from Missouri and Ohio in Jan. 2003 may be too conservative (Table 1). This potential cause for underestimates does not apply to Apr. 2004, however, because survival was tested at temperatures lower than the LST we subsequently determined. Future examination of variation in depth of hardiness in response to ambient conditions in field trials covering a wide geographic area may be one way to reconcile the LST estimates for common buckthorn that are higher than the record daily minima with the extensive climatic range of the region it has invaded. It has been long known that the second stage of cold hardening is dependent on ambient winter temperatures (Weiser, 1970) and that the degree of cold hardiness can differ throughout the winter (Levitt, 1980), from year to year

(Taulavuori et al., 2004), or among locations (Schrader and Graves, 2003). Actual minimum temperatures dropped below the LST of common buckthorn in New Franklin in Jan. and Feb. 2004 and below that of carolina buckthorn in January and February of both years (Figs. 1 and 2). It appears, though, that cold hardiness increased in both species based on their survival in April of both years (Figs. 1 and 2). Cold hardiness increases as the temperature slowly lowers and it decreases as the temperature increases (Levitt, 1980), which is referred to by Weiser (1970) as the transient third stage of cold-hardening. Future work in assessing the cold hardiness of carolina buckthorn should include field trials that include numerous plant provenances and that span more than one season at multiple sites to account for variations among genotypes and in climatic patterns.

Regardless of the region in which selections of carolina buckthorn are cold hardy, questions regarding its potential invasiveness merit attention. We drew on conclusions from previous research to question how the timing of vernal bud break of common buckthorn, a known invasive species, compares with the timing of bud break of carolina buckthorn. The delayed bud break of carolina buckthorn relative to that of common buckthorn provides one line of evidence to support the contention that invasiveness potential of carolina buckthorn is comparatively low. Early bud break of common buckthorn contributes to its invasiveness (Harrington et al., 1989). Many understory plants in the spring accumulate carbon reserves before overstory canopy closure (Routhier and Lapointe, 2002). Even with leaf mulch, which insulated the soil (Table 3), the FDBB of carolina buckthorn was later than that of common buckthorn. We have observed that carolina buckthorn is typically found in understory habitats in its native range. Harrington et al. (1989) concluded that early bud break of common buckthorn in a Wisconsin forest helped promote its persistence in an

understory habitat, but not in an open habitat. This effect was manifest in the reduced fecundity of native shade-intolerant annuals in the presence of *Lonicera maackii* (Rupr.) Herder in southern Ohio forests (Gould and Gorchov, 2000). Early emergence of leaves of *P. australis* and *T. angustifolia* contributed to their invasiveness in brackish and freshwater tidal marshes in Connecticut (Farnsworth and Meyerson, 2003).

Vernal phenology of carolina buckthorn appears to be controlled by environmental factors such as air temperature (Hanninen, 1991) than by developmental constraints; both RATE and total percentage of broken buds were greater for mulched carolina buckthorns than for those in the non-mulched treatment. Augspurger (2004) found that the vernal phenology of seedlings of *Aesculus glabra* Willd. was affected more by temperature cues than by ontogenic regulation. Although documenting the leaf longevity of carolina buckthorn during the entire growing season would be valuable, our data on vernal bud break represent an important component of the information useful for predicting the invasiveness of carolina buckthorn. Timing of bud break of carolina buckthorn indicates that it might be less invasive than common buckthorn, but other factors also need to be considered before promoting its use in the landscape and nursery industries. These factors include fruit set and dispersal (Godwin, 1936; Kollmann and Pirl, 1995), seed germinability (Stewart and Graves, 2005), and vigorous plant development (Archibold et al., 1997).

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Table 1. Lowest survival temperatures (°C) of 2-year-old *Rhamnus cathartica* (common buckthorn) and *Rhamnus caroliniana* (carolina buckthorn) native to Missouri, Ohio, and Texas, and established in Ames, Iowa. Values for species are means of three and nine replications for *Rhamnus cathartica* and *Rhamnus caroliniana*, respectively, at each assessment date. Values partitioned by provenance are means of three replications. Survival percentages were based on visual assessments of stem viability on 2 Apr. Thirty-year normal daily minimum temperature data for Ames, Iowa, for each month are provided with the 50-year-record daily minimum temperature in parentheses.

	Date 2002-03			Survival
Species and population	5 Oct.	11 Jan.	2 Apr.	(%)
Rhamnus cathartica	-13B[z]	-24A	-17A	100
Rhamnus caroliniana	-5A	-21A	-12A	67
Missouri	-5a[y]	-24a	-21c	100
Ohio	-7a	-24a	-13b	100
Texas	-3a	-16a	0a	0
Daily minimum temperature	7 (-2)	-13 (-28)	1 (-8)	

[z]Species means within each column followed by the same capital letter are not different at $P < 0.05$ according to Holm's method.

[y]Population means within each column followed by the same lower-case letter are not different at $P < 0.05$ according to Holm's method.

Table 2. Lowest survival temperatures (°C) of *Rhamnus cathartica* (common buckthorn) and *Rhamnus caroliniana* (carolina buckthorn) native to Missouri and Texas, and established in Ames, Iowa and New Franklin, Mo. Values for species within location are means of four and eight replications for *R. cathartica* and *R. caroliniana*, respectively, on 16 Oct. Values across location on 16 Oct. were not combined due to an interaction between species and location. Values for species are means of eight and 16 replications for *R. cathartica* and *R. caroliniana*, respectively, on 14 Jan. and 12 Apr. Values partitioned by provenance are means of eight replications. Survival percentages were based on visual assessment of stem viability on 12 Apr.. Thirty-year normal minimum temperature data for each month for Ames, Iowa and New Franklin, Mo. are provided are provided with the 50-year-record minimum temperature in parentheses.

Species and population	Date 2003-04				Survival (%)	
	16 Oct.		14 Jan.	12 Apr.	Ames	New Franklin
	Ames	New Franklin				
Rhamnus cathartica	-11A[z]	-15B	-24B[y]	-4A	100	100
Rhamnus caroliniana	-8A	-1A	-17A	-1A	59	100
Missouri	-6a[x]	-6a	-16a	-1a	100	100
Texas	-2a	-2a	-18a	-1a	17	100

Daily minimum temperature

Ames	5 (-4)		-13 (-29)	3 (-7)
New Franklin		7 (-2)	-8 (-23)	6 (-6)

[z]Location means within species within the 16 Oct. columns followed by the same capital letter are not different at $P < 0.05$ according to Holm's method.

[y] Species means within each column followed by the same capital letter are not different at $P < 0.05$ according to Holm's method.

[x]Population means within each column followed by the same lower-case letter are not different at $P < 0.05$ according to Holm's method.

Table 3. Mean daily minimum soil temperature with and without mulch, respectively, for Nov. 2001 through Apr. 2002, in plots established to evaluate bud break of 1-year-old plants of *Rhamnus cathartica* (common buckthorn) and *Rhamnus caroliniana* (carolina buckthorn) from Missouri, Ohio, and Texas.

	Date (2001-02)					
Soil temperature (°C)	November	December	January	February	March	April
Mulch	11.4	7.8	3.0	3.5	3.3	9.2
No mulch	6.1	0.4	-2.0	0.2	0.0	8.5

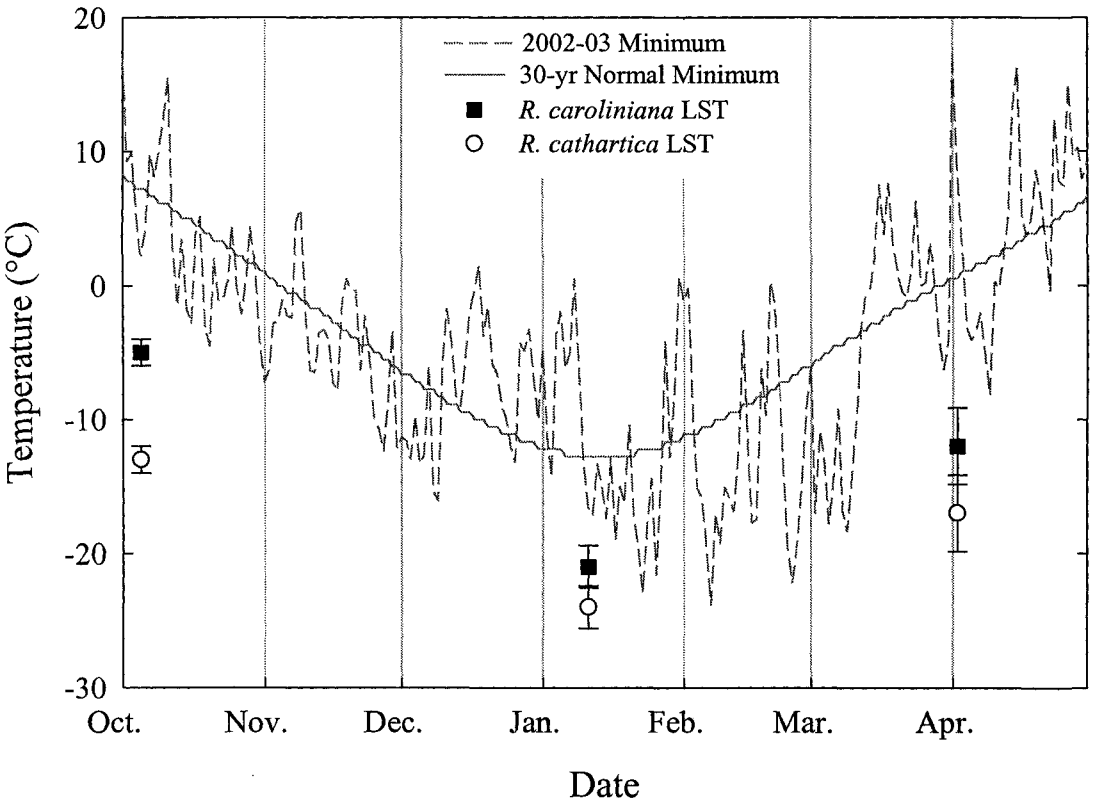
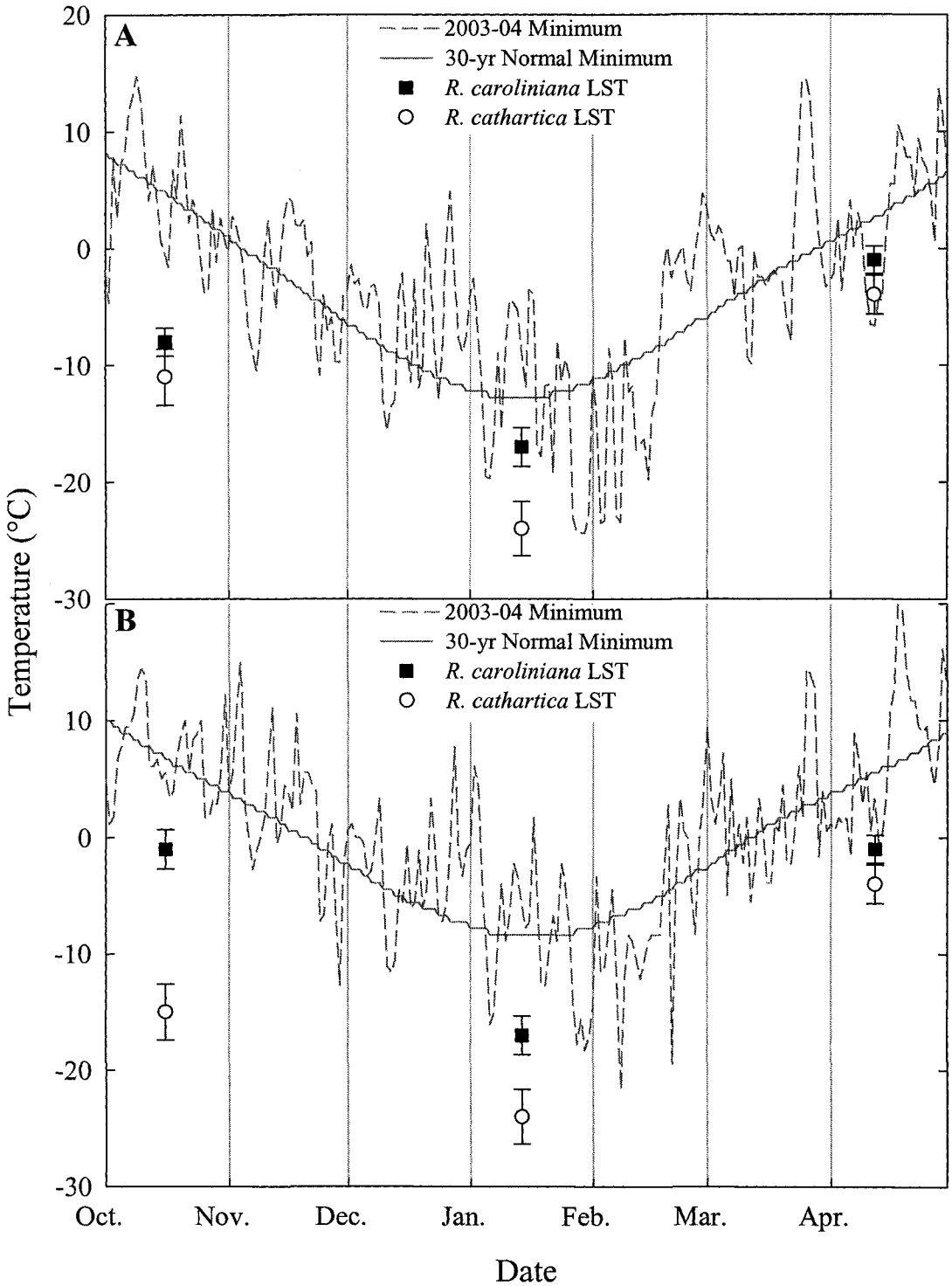


Fig. 1. Daily miminum temperatures for 2002-03 and 30-year normal minimum temperatures in Ames, Iowa, for October through April in addition to the lowest survival temperatures (LST) of 2-year-old *Rhamnus caroliniana* and *Rhamnus cathartica*, which were assessed on 5 Oct. 2002 and 11 Jan. and 2 Apr. 2003. Data points are mean values ± SE.


A
B
2003-04 Minimum
30-yr Normal Minimum
R. caroliniana LST
R. cathartica LST
Temperature (°C)
20
10
0
-10
-20
-30
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
Date

CHAPTER 5. PHOTOSYNTHESIS, GROWTH, CARBON ALLOCATION, AND FRUIT LOAD OF *FRANGULA CAROLINIANA* AND *RHAMNUS CATHARTICA*

A paper to be submitted to Oecologia

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Key words Invasive species, growth analysis, *Rhamnus caroliniana*, specific leaf area, net assimilation rate

Abstract Typical of many invasive woody species, *Rhamnus cathartica* tolerates various degrees of soil moisture and produces large numbers of fruit annually. Little information, however, is available regarding the ecophysiological performance of this species under optimal field conditions. Not only is this information necessary to understand which functional traits (i.e., relative growth rate, specific leaf area, and season-long water-use efficiency) enhance its success in disturbed environments, but to also help predict the invasiveness of closely related species such as *Frangula caroliniana*, which may be introduced through human activities. We hypothesized that *R. cathartica* would have greater relative growth rate, specific leaf area, and water-use efficiency over time than would *F. caroliniana*. We also predicted that fecundity as defined by fruit count per unit leaf area and branch length would be higher for *R. cathartica* than for similarly aged plants of *F. caroliniana*. Photosynthesis, growth, and carbon allocation patterns of seedlings of both species were studied over time under modified field conditions. Although relative growth rate of *R. cathartica* was initially greater than that of *F. caroliniana*, there were no subsequent differences in plants after the 14-day harvest. Specific leaf area of *R. cathartica*

was greater than that of *F. caroliniana* at the 98-day harvest, but there was not a strong relationship between specific leaf area and relative growth rate for either species (*F. caroliniana*: $r^2 = 0.14$, *R. cathartica*: $r^2 = 0.13$). There was a stronger relationship, however, between relative growth rate and net assimilation rate for both species (*F. caroliniana*: $r^2 = 0.46$, *R. cathartica*: $r^2 = 0.30$). Net photosynthetic rate of *R. cathartica* was higher than that of *F. caroliniana* after the 42-day harvest, but there were no differences in total plant dry weight after the 28-day harvest. Length, leaf surface area, and fruit count of two-year-old branches of *F. caroliniana* and *R. cathartica* were sampled in the field and measured to determine the fecundity of each species. After accounting for branch length and leaf surface area, the fruit count of *F. caroliniana* was only 41% of that of *R. cathartica*. We conclude that, under favorable field conditions, seedlings of *R. cathartica* and *F. caroliniana* appear to establish similarly, although there were differences in growth and photosynthetic patterns over time. We also conclude that *R. cathartica* appears to have a greater fecundity than *F. caroliniana*.

Introduction

Although patterns of ecological succession in naturally disturbed habitats have led to so-called invasions by pioneering species (e.g., *Alnus* species) over the past several hundred years (Bazzaz 1986), most invasions in disturbed environments (Daehler 2003) have been due to plants introduced through human activities (Reichard 1997; Reichard and White 2001). One invasive species representative of this pattern, *Rhamnus cathartica* L. (common or European buckthorn), has been in North America for at least 200 years (Wyman 1971). It was originally introduced in the early 1800s and was used as a shelterbelt and hedge plant throughout the eastern United States and eastern maritime provinces of Canada (Gourley

1985). *Rhamnus cathartica* quickly escaped cultivation, however, and has invaded several natural communities throughout the northern United States and southern Canada (Gourley 1985; Archibold et al. 1997).

Like many other invasive species, some traits that make *R. cathartica* a successful invader include its fecundity (Archibold et al. 1997; Kollmann and Grubb 1999), high tolerance to extremes in soil moisture (Stewart and Graves 2004), and its low susceptibility to herbivory and pathogens (Billington 1949). Such information would be useful in predicting the invasive potential of species closely related to *R. cathartica*, including *Frangula caroliniana* (Walt.) Gray (Carolina buckthorn), a North American small tree or shrub. Indeed, a powerful method to assess the invasive characteristics of a species is to make comparisons between ecologically and taxonomically similar invasive and native taxa (Pattison et al. 1998; Morris et al. 2002). Similar to naturalized specimens of *R. cathartica*, *F. caroliniana* is frequently found in disturbed areas at the edge of woodlands in its native range (personal observation). And like that of *R. cathartica* (Godwin 1943; Kollmann and Pirl 1995; Archibold et al. 1997), the fruit of *F. caroliniana* is eaten and spread by frugivores (Nokes 2001), a trait likely to be exploited if the species naturalizes elsewhere. Due to increased horticultural interest in *F. caroliniana* (Nokes 2001; Graves 2002; Stewart and Graves 2004), concerns about its invasive potential need to be addressed.

Although tolerance to water stress of both species has been evaluated (Stewart and Graves 2004), very little work has been done to evaluate how ecophysiological characteristics such as juvenile growth, leaf characteristics, carbon allocation patterns as related to seedling age, and season-long resource-use efficiency contribute to the known invasive potential of *R. cathartica* and the likelihood of invasiveness of *F. caroliniana*.

While Harrington et al. (1989) found that 29% of annual carbon gain of *R. cathartica* in a Wisconsin forest occurred before leaf emergence of *Cornus racemosa* Lam., a more comprehensive view is needed to determine its ontogenical pattern of carbon allocation over the growing season. Research is also warranted to determine if leaf characteristics of *R. cathartica* enable it to assimilate carbon more efficiently than surrounding plants or related taxa such as *F. caroliniana*. Successful invaders in seasonal environments usually exhibit greater rates of resource utilization when resources are available (Stratton and Goldstein 2001). Invasive species also typically have higher specific leaf area and photosynthetic rates than native species (Allison and Vitousek 2004). Specific leaf area is important to plant growth because it leads to a high proportion of leaf area per unit dry mass invested (i.e., efficient light capture) and high photosynthetic capacity (Reich et al. 1997). Higher photosynthetic rates may also lead to higher rates of biomass accumulation and growth (Lambers and Poorter 1992).

The reproductive biology of invaders frequently contributes to their ecological advantage over native flora (Bazzaz 1986; Honig et al. 1992; Marco and Páez 2000). Likewise, the fecundity of *R. cathartica* has repeatedly been implicated in contributing to its aggressive spread (Gourley 1985), but apart from the work of Gourley (1985), little has been done that specifically characterizes the reproductive capacity of mature, established plants of this species.

We hypothesized that under field conditions, seedlings of *R. cathartica* would have greater specific leaf area than that of *F. caroliniana*, which would result in comparatively higher growth rates. We also predicted that *R. cathartica* would have greater season-long water-use efficiency under conditions of high soil water content than *F. caroliniana*.

Although Kramer and Boyer (1995) considered the measure of CO_2 and H_2O exchange of individual leaves to be a useful approximation of water-use efficiency, they considered the ratio of dry matter produced per amount of water used to be more accurate. We also postulated that fruit count per unit leaf area and branch length of mature *R. cathartica* plants would be greater than that of similarly aged *F. caroliniana* plants.

Materials and Methods

Photosynthesis, growth, and carbon allocation. Seeds of *F. caroliniana* were collected in October 2002 from four plants in Brazito, Mo. (lat. 38°26'44"N; long. 92°18'9"W), and four plants in Kerrville, Texas (lat. 30°02'50"N; long. 99°08'24"W). Seeds of *R. cathartica* also were collected in October 2002 from four plants in naturalized populations in Ames, Iowa (lat. 42°2'5"N; long. 93°37'11"W). All seeds were stratified for 63 d at 4 °C (Stewart and Graves, 2005) and germinated in a greenhouse.

To insulate the roots of potted seedlings from solar radiation in the field, we inserted 168 14.6-L containers (Model C-2000; Nursery Supplies, Chambersburg, Pa.) into 21.9-L containers (model C-2800; Nursery Supplies) and sprayed polyurethane liquid foam (II-600 Handi-Foam Slow Rise; Fomo Products, Norton, Ohio) into the space between sidewalls of the two containers. Five holes (diameter = 1.3 cm) were drilled into the bottom of the modified containers to allow for drainage. The exterior of the containers were then wrapped with aluminum foil.

On 18 Apr. 2003, 12 soil cores (length = 30.5 cm, diameter = 2.5 cm) were sampled and then analyzed for levels of nitrogen, phosphorus, potassium, calcium, magnesium, and organic matter at the Soil and Plant Analysis Laboratory of Iowa State University.

To simulate field conditions, 168 intact Webster (fine-loamy, mixed, superactive, mesic Typic Endoaquoll) soil cores (length = 30.5 cm, diameter = 30.5 cm) were dug out of a field at a research farm north of Ames, Iowa. The soil cores were then inserted into 14.6-L containers (model C-2000; Nursery Supplies), which were then inserted into the modified containers. In total, there were 168 seedlings (112 *F. caroliniana* [56 per provenance, 14 per sibling group] and 56 *R. cathartica* [14 per sibling group]) that were planted, one per container, on 14 June 2003. The plants were held near the field site in a shaded hoophouse until they were randomly arranged on a field plot north of Ames, Iowa, on 27 June 2003. Plywood lids (width =30.5 cm, length = 30.5 cm, depth = 0.9 cm) with closed-cell polyethylene foam gaskets with holes (diameter = 3.2 cm) in the middle were secured on the top of each container to minimize soil evaporation. Open space between the holes and plant stems were covered with duct tape to minimize soil evaporation further. Each plant was fertilized with 1 L of a solution of a mixture of Peters Excel All-Purpose and Cal-Mag (16.5N-2.2P-13.5K) (Scotts, Marysville, Ohio) that contained N at 11 mM on 1 July, 25 July, and 29 Aug., 2003. Containers were weighed with an electronic balance every 2 to 3 d beginning on 2 July 2003. Twenty-four seedlings (16 *F. caroliniana* [eight per provenance, two per sibling group] and eight *R. cathartica* [two per sibling group]) were randomly chosen and harvested on 3 July 2003 to serve as baseline data for growth analysis calculations. Sixteen *F. caroliniana* (eight per provenance) and eight *R. cathartica* were harvested every 14 d (16 July, 30 July, 13 Aug., 27 Aug., 10 Sept., 24 Sept., and 9 Oct. 2003). After cleaning the roots of each plant, the roots were stored in a 25% isopropyl alcohol solution at 4 °C. Root length was then measured (Kaspar and Ewing 1997). Root surface area was calculated by assuming roots were cylindrical. Leaf surface area was measured with a leaf area meter

(model 3100; LI-COR, Lincoln, Nebr.). Dry mass of roots, stems, and leaves were determined after each harvest. All tissues were dried 48 h at 67 °C. We also determined relative growth and net assimilation rates (Harper, 1977), root-to-shoot ratio (g/g), root-to-leaf ratio (g/g and cm^2/cm^2), leaf area ratio, leaf mass ratio, and specific leaf area. Water-use efficiency per harvest period was calculated as the ratio between total plant dry mass and total evapotranspiration.

Net photosynthetic rate of the youngest fully expanded leaf of the longest stem of each plant was measured with a photosynthesis system (LI-6400; LI-COR) every 14 d at midday beginning on 10 July 2003. The first measurements on 10 July were done with a clear-top leaf chamber, but the remaining measurements on 24 July, 7 Aug., 21 Aug., 4 Sept., 18 Sept., and 2 Oct. 2003 were done with a LED light source (model 6400-02B; LI-COR) that was set at 1500 $\mu mol/m^2/s$.

We used a data logger (model CR23X; Campbell Scientific, Logan, Utah) equipped with a air temperature/relative humidity probe (model CS500; Campbell Scientific), pyranometer (model PYR; Apogee Instruments, Logan, Utah), six soil temperature probes (model 107; Campbell Scientific) to determine mean air temperature, relative humidity, incoming shortwave radiation, and soil temperature in the field and containers (Table 1). Precipitation was measured with a tipping bucket rain gauge (model TE525; Texas Electronics, Dallas, Texas) on a weather station 2000 m east of the field plot.

Analyses were conducted with the PROC GLM procedure in SAS/STAT® version 8.2 (SAS Institute, Cary, N.C.). Comparisons of means were done using Tukey's honestly significant difference test. Regression analysis was performed on measured parameters to test for linear and quadratic responses.

Fruit load. Fifty 2-year-old, physiologically mature branches were collected *ad libitum* from 10 *R. cathartica* trees of similar size and shape on 11 Sept. (one tree), 18 Sept. (four trees), 25 Sept. (four trees), and 1 Oct. 2004 (one tree) located in naturalized populations in Ames, Iowa (lat. 42°2'5"N; long. 93°37'11"W). On 12 Oct. 2004, fifty branches of varying lengths were collected *ad libitum* from 10 *F. caroliniana* trees of similar size and shape in a native population at the Washington University Tyson Research Center in Missouri (lat. 38°32'13"N; long. 90°33'42"W). Collection was staggered to collect only ripe fruit of both species. After the branches were collected, we measured their length, number of fruits, and total leaf surface area.

Least squares regression analysis was conducted for both species with the PROC MIXED procedure in SAS/STAT (SAS Institute) to estimate the relationship between the dependent variable, fruit number, and the independent variables, branch length and leaf area. Fruit count was log-transformed to stabilize the variance. Each tree was considered a random block effect in the model because the selected trees were a sample of the entire population.

Results

Photosynthesis, growth, carbon allocation. The plant dry weight of *R. cathartica* was 2.1 times greater than that of *F. caroliniana* at the 14-d harvest (Table 2). It was 46% greater than that of *F. caroliniana* at the 28-d harvest, but there were no differences between the species thereafter (Table 2). Plant dry weight increased linearly over time for both *F. caroliniana* ($P < 0.0001$, $r^2 = 0.61$) and *R. cathartica* ($P < 0.0001$, $r^2 = 0.67$). Stem dry weight of *R. cathartica* was twice as great as that of *F. caroliniana* at the 14-, 28-, and 42-d harvests, and 54% greater at the70-d harvest (Table 2).

The leaf area of seedlings of *R. cathartica* harvested after 14 d was 69% greater than that of *F. caroliniana* seedlings, but there were no other differences (Table 2). Root dry weight of *R. cathartica* was 63% and 46% greater than that of *F. caroliniana* at the 14- and 98-d harvests, respectively (Table 2). Although root length of *R. cathartica* was greater than that of *F. caroliniana* after 14 d, there were no other differences in root length (Table 2). Root surface area of *R. cathartica* was 77% greater than that of *F. caroliniana* at the 14-d harvest, but there were no differences between species thereafter (Table 2).

The area-based root-to-leaf ratio of *F. caroliniana* was 44% greater than that of *R. cathartica* at the 28-d harvest, but the ratio of *R. cathartica* was 45%, 62%, and 2.7 times greater than that of *F. caroliniana* at the 70-, 84-, and 98-d harvests, respectively (Table 2).

Relative growth rate of *R. cathartica* was 2.1 times greater than that of *F. caroliniana* at the 14-d harvest, but there were no subsequent differences between the species (data not shown). Net assimilation rate of *R. cathartica* was 1.9 times, 30%, and 41% greater than that of *F. caroliniana* at the 14-, 84-, and 98-d harvests, respectively (Table 2).

Leaf area ratio of *F. caroliniana* was 29%, 28%, 56%, and 67% greater than that of *R. cathartica* at the 56-, 70-, 84-, and 98-d harvests, respectively (Table 2). The specific leaf area of *R. cathartica* was 11% and 12% greater than that of *F. caroliniana* at the 42- and 98-d harvests, respectively (Table 2). Leaf mass ratio of *F. caroliniana* was 10%, 18%, 28%, 36%, 65%, and 87% greater than that of *R. cathartica* at the 28-, 42-, 56-, 70-, 84-, and 98-d harvests, respectively (Table 2). Root mass ratio of *R. cathartica* was 21%, 19%, and 27% greater than that of *F. caroliniana* at the 70-, 84-, and 98-d harvests, respectively (Table 2). Stem mass ratio of *R. cathartica* was 25%, 33%, 30%, 17%, 29%, and 24% greater than that of *F. caroliniana* at the 28-, 42-, 56-, 70-, 84-, and 98-d harvests, respectively (Table 2).

There was not a strong correlation between relative growth rate and specific leaf area for either species (*F. caroliniana*: y = 0.0193 + 0.0002[specific leaf area], $r^2 = 0.13$; *R. cathartica*: y = 0.008 + 0.0003[specific leaf area], $r^2 = 0.14$) or between relative growth rate and leaf area ratio (*F. caroliniana*: y = 0.0287 + 0.0003[leaf area ratio], $r^2 = 0.10$; *R. cathartica*: y = 0.025 + 0.0005[leaf area ratio], $r^2 = 0.20$). There was a stronger correlation, however, between relative growth rate and net assimilation rate for both species (*F. caroliniana*: y = 0.0126 + 0.006[net assimilation rate], $r^2 = 0.46$; *R. cathartica*: y = 0.0117 + 0.0056[net assimilation rate], $r^2 = 0.30$) (Fig. 1).

Although net photosynthetic rate of *F. caroliniana* was 18% and 39% greater than that of *R. cathartica* at the 14- and 28-d harvests, respectively, net photosynthetic rate of *R. cathartica* exceeded that of *F. caroliniana* by 13%, 24%, 33%, and 39% at the 42-, 56-, 70-, and 84-d harvests, respectively (Fig. 2). Net photosynthetic rate of *R. cathartica* was three times greater than that of *F. caroliniana* at the 96-d harvest (Fig. 2). After 14 d, water-use efficiency of *R. cathartica* (16.7 g/L) was greater than that of *F. caroliniana* (7.1 g/L) ($P = 0.01$), but there were no subsequent differences in water-use efficiency (data not shown).

Fruit load. The log(fruit number) of *F. caroliniana* was 1.56 log(fruit) and the log(fruit number) of *R. cathartica* was 2.44 log(fruit). Hence, mean fruit number of *F. caroliniana* was only 41% ($P = 0.0027$) of that of *R. cathartica* at an average branch length of 19.8 cm and average leaf area of 136.3 cm^2 (*F. caroliniana*: log[fruit number] = 1.04 + 0.02[branch length] + 0.001[leaf area]; *R. cathartica*: log[fruit number] = 1.59 + 0.03[branch length] + 0.002[leaf area]).

Discussion

In nutrient-rich and productive habitats, high specific leaf area improves the competitive ability of a species (Poorter 1990) and is strongly correlated with high relative growth rate of several species (Reich et al. 1997). The higher relative growth rate of fast-growing species in comparison with slow-growing ones in similar growing conditions has been largely explained by differences in a component of leaf area ratio, specific leaf area (Lambers et al. 1998). Invasive species capture resources more efficiently and have higher growth rates than native species due to their higher specific leaf area (Baruch and Goldstein 1999), which is the quotient of leaf area ratio divided by leaf mass ratio (Evans 1972), and relative growth rate (Pattison et al. 1998), which is the product of leaf area ratio and net assimilation rate (Evans 1972).

The lack of differences in specific leaf area between *F. caroliniana* and *R. cathartica* appears to account for the similarity in relative growth rate for most of the growing period (Table 2). The poor correlation between specific leaf area and relative growth rate for both species may be due to the exposed conditions of the study. Plants with high specific leaf area are typically found in dense vegetation (Poorter 1990). A strong correlation was found, though, between net assimilation rate and relative growth rate (Fig. 1). Although net assimilation rate is considered poorly correlated with relative growth rate for many species (Lambers et al. 1998), high net assimilation rate is considered a functional trait of successful invaders (Pattison et al. 1998; Grotkopp et al. 2002). The relatively high net assimilation rate of *R. cathartica* may explain the comparatively high relative growth rate of *R. cathartica* at the 14-d harvest. Net assimilation rate is defined simply as an increase in dry weight per unit leaf area, but is a complex interaction of physiology, biomass allocation, and leaf area

formation (Lambers and Poorter 1992). Although leaf area ratio and its component, specific leaf area, were similar between species at the 14-d harvest, leaves of *R. cathartica* appear to be more efficient at fixing carbon, confirming conclusions in an earlier study (Stewart and Graves 2004), even though the instantaneous photosynthetic rate of *F. caroliniana* was greater than that of *R. cathartica* at the 14-d harvest (Fig. 2). Specific leaf area of *R. cathartica* seedlings at the 98-d harvest, however, was greater than that of *F. caroliniana*, but there were no differences in relative growth rate. After 56 d, the leaf area ratio of *R. cathartica* was not only consistently less than that of *F. caroliniana*, but as time progressed, the magnitude of the difference between species increased. Leaf mass ratio of *R. cathartica* followed a similar pattern in difference and increasing magnitude, but began after 28 d. The difference in specific leaf area and shift in allocation patterns may be best explained by the difference in leaf mass ratio between seedlings of *R. cathartica* and *F. caroliniana* at the 98-d harvest. The leaf area ratio of *R. cathartica* was 40% less than that of *F. caroliniana*, but the leaf mass ratio of *R. cathartica* was 46% less than that of *F. caroliniana*, which resulted in a higher specific leaf area for *R. cathartica* relative to that of *F. caroliniana*. Although younger, distal leaves of *R. cathartica* shaded out and may have caused the discharge of older leaves, the root dry weight of *R. cathartica* tended consistently to be greater than that of *F. caroliniana*, though the difference was not significant until the 98-d harvest. This finding, along with the findings that after 70 d, area-based root-to-leaf ratio and root weight ratio of *R. cathartica* were greater compared to those measures of *F. caroliniana*, leads us to suggest that while there is a possibility that the higher specific leaf area of seedlings at the 98-d harvest may contribute to the invasiveness of *R. cathartica* under more stressful conditions,

fitness of young seedlings of both species, as defined by total plant dry weight, appears to be similar, though by different means or strategies.

Increases in root mass ratio with simultaneous decreases in leaf mass ratio is an evolved functional trait in some species that have adapted to low nutrient and/or water availability (Walters et al. 1993). If young seedlings of both species were under nutrient or drought stress, the difference in carbon allocation to roots may be considered a response to either nutrient deficiency or low water availability (Monneveux and Belhassen 1996), but we believe that was unlikely given the conditions of the experiment. A more plausible explanation for the difference in root carbon allocation is the genetic constraint imposed on the allocation patterns of *R. cathartica* seedlings. The relatively high area-based root-to-leaf ratio of *R. cathartica* may not be advantageous during periods of high water and nutrient availability, but it would become beneficial during periodic episodes of drought. Increases in root mass ratio may be related to a gradual accumulation of support tissue manifest in the root and stem organs that accompanies increases in age (Walters et al. 1993). Indeed, the rise in root mass ratio of *R. cathartica* over time was concomitant with higher stem mass ratio of *R. cathartica* after 70 d.

Although total stem length did not differ between species during most of the study, stem mass ratio of *R. cathartica*, which was greater than that of *F. caroliniana* after 28 d, not only suggests more structural support to developing seedlings, but also the possible storage of extractable proteins for subsequent mobilization to support biosynthesis (Sauter and Van Cleve 1990). In a comparison of 80 woody species from Great Britain and northern Spain, there was a trade-off between investment in foliage versus stems (Cornelissen et al. 1996). While stem mass ratio of *R. cathartica* remained relatively constant over time, the higher

stem dry weights of 14-, 28-, 42-, and 84-d-old seedlings of *R. cathartica* relative to that of *F. caroliniana* typified the differences in carbon allocation to stems of both species throughout the study. In its native range, *R. cathartica* is an understory shrub that is typically found along forest edges (Godwin 1943). Carbon allocation to stems is typical of shade-tolerant plants where elongation and structural support are critical for efficient harvesting of light in the understory (Dale and Causton 1992). Carbon investment in the stems may also make *R. cathartica* more resilient to damage or attack by herbivores and pathogens (Niinemets 1998).

Rhamnus cathartica appears to be more efficient in terms of water-use efficiency and was more aggressive in growth (i.e., relative growth rate and total plant dry weight) earlier in its development than *F. caroliniana*. It is not surprising, however, that there were no differences in water-use efficiency for the remainder of the study. When water is abundant, high water-use efficiency leads to relatively low productivity (Meinzer et al. 1992) and can put plants at an ecological disadvantage in environments with seasonal variation in water availability (DeLucia and Heckathorn 1989). An important factor in the success of invasive species is phenotypic plasticity (Stratton and Goldstein 2001). *Rhamnus cathartica* and *F. caroliniana* maintain growth over a wide range of soil water contents (Stewart and Graves 2004), but further work needs to be done to determine if the water-use efficiency of both species varies depending on whether plants are exposed to drought, optimal conditions, or to saturation and flooding. Furthermore, biomass allocation patterns and functional traits are not constant during plant ontogeny (Whittaker 1962; Niinemets 1998). Not only is it important to understand the establishment phase of young seedlings, but also mature, established plants that are capable of sustaining gene flow via fruit and seed formation.

Copious fruit production is a well-known trait of invasive species. The high fecundity of *Sapium sebiferum* (L.) Roxb. (Chinese tallow tree) (Renne et al. 2000) and *Ligustrum sinense* Lour. (Chinese privet) (Morris et al. 2002) have contributed to their invasive spread throughout the southeastern United States. Although our work does not provide an estimate of the total fruit count per plant, it provides a relative measure of fecundity that suggests that *F. caroliniana* may not be invasive or at least not on the same magnitude as *R. cathartica*. We recognize that fruit production of individual plants is highly variable (Whittaker 1962), particularly from year to year. Further work needs to be done over multiple years to confirm our initial conclusion that the fruit production of *F. caroliniana* is nearly less than half of that of *R. cathartica*.

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Table 1. Mean monthly values of air temperature, relative humidity, and incoming shortwave radiation during the study period in 2003. Precipitation is listed in total monthly amounts.

	Date			
Parameter	July	August	September	October
Air temperature (°C)	21.1	22.0	15.6	14.1
Relative humidity (%)	64	61	54	46
Incoming shortwave radiation (W/m^2)	417.8	398	341.7	355.4
Precipitation (mm)	102.6	20.3	66.8	15.5

Table 2. Comparisons of seedlings of *Frangula caroliniana* (Walt.) Gray and *Rhamnus cathartica* L. harvested every 14 d over a 98-d period. Values for species are means of 16 and eight replications of *F. caroliniana* and *R. cathartica*, respectively. The dependent variable in all regressions is time in days.

	Date (d)							Significance		
Dependent variable and species	14	28	42	56	70	84	98	Linear	Quadratic	r^2
Plant dry weight (g)										
Frangula caroliniana	0.71 B[a]	1.29 B	2.66 A	4.53 A	7.33 A	9.08 A	9.11 A	***[b]	NS	0.61
Rhamnus cathartica	1.50 A	1.92 A	3.44 A	4.70 A	8.41 A	10.08 A	9.69 A	***	NS	0.67
Evapotranspiration (L)										
Frangula caroliniana	0.10 A	0.46 A	0.95 A	1.83 A	2.71 A	3.36 A	3.36 A	***	NS	0.83
Rhamnus cathartica	0.09 A	0.55 A	1.03 A	1.77 A	2.72 A	3.58 A	3.94 A	***	NS	0.92
Water-use efficiency (g/L)										
Frangula caroliniana	7.1 B	3.0 A	2.7 A	2.4 A	2.5 A	2.6 A	2.6 A	**	**	0.15
Rhamnus cathartica	16.7 A	3.5 A	3.1 A	2.6 A	3.1 A	2.8 A	2.5 A	***	***	0.47
Stem dry weight (g)										
Frangula caroliniana	0.20 B	0.30 B	0.63 B	1.07 A	1.75 A	2.02 B	2.33 A	***	NS	0.56
Rhamnus cathartica	0.42 A	0.62 A	1.23 A	1.57 A	2.46 A	3.12 A	3.17 A	***	NS	0.63
Shoot dry weight (g)										

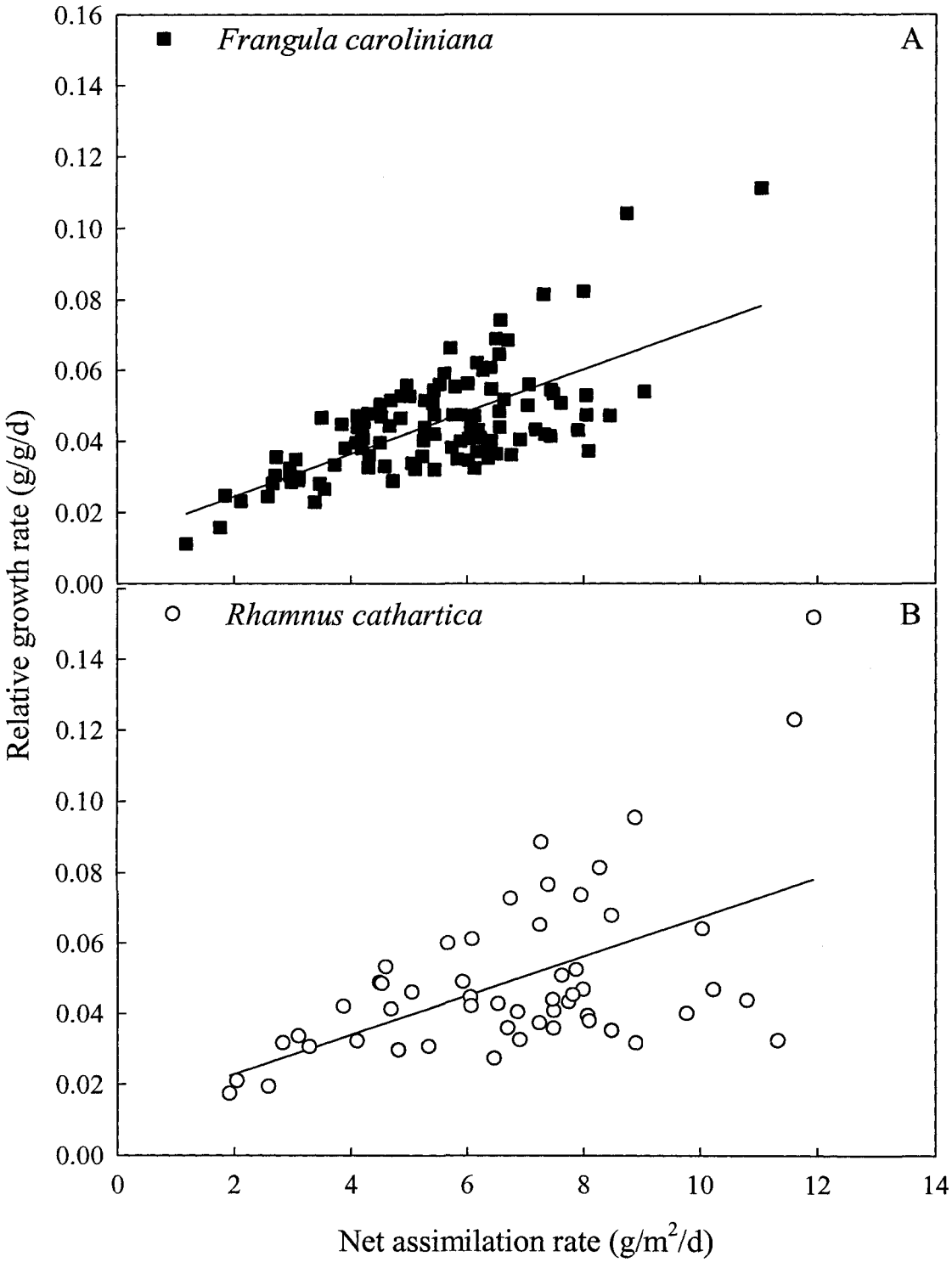
Frangula caroliniana	0.56 B	0.98 B	2.05 A	3.33 A	5.26 A	5.78 A	6.19 A	***	NS	0.58
Rhamnus cathartica	1.19 A	1.54 A	2.73 A	3.38 A	5.43 A	5.75 A	5.41 A	***	NS	0.55
Leaf area (cm^2)										
Frangula caroliniana	56.0 B	107.8 A	202.6 A	282.1 A	381.9 A	374.2 A	366.1 A	***	**	0.48
Rhamnus cathartica	94.8 A	150.1 A	235.4 A	224.4 A	329.6 A	281.0 A	237.5 A	***	**	0.34
Root dry weight (g)										
Frangula caroliniana	0.16 B	0.30 A	0.60 A	1.20 A	2.07 A	3.30 A	2.93 B	***	NS	0.59
Rhamnus cathartica	0.26 A	0.38 A	0.71 A	1.32 A	2.98 A	4.33 A	4.28 A	***	**	0.75
Root length (cm)										
Frangula caroliniana	499 B	1188 A	1740 A	3563 A	5279 A	7359 A	5660 A	***	NS	0.38
Rhamnus cathartica	1018 A	1400 A	2071 A	3478 A	7063 A	8667 A	8689 A	***	NS	0.51
Root surface area (cm^2)										
Frangula caroliniana	70.6 B	157.2 A	253.1 A	489.9 A	775.1 A	1049.6A	825.8 A	***	NS	0.43
Rhamnus cathartica	125.1 A	159.4 A	269.6 A	443.8 A	943.3 A	1158.3 A	1134.8 A	***	NS	0.57
Root-to-shoot ratio (g/g)										
Frangula caroliniana	0.27 A	0.30 A	0.30 A	0.35 A	0.38 B	0.55 B	0.49 B	***	NS	0.34
Rhamnus cathartica	0.25 A	0.25 A	0.25 A	0.37 A	0.53 A	0.77 A	0.82 A	***	**	0.75
Root-to-leaf ratio (cm^2/cm^2)										
Frangula caroliniana	1.22 A	1.48 A	1.28 A	1.71 A	1.86 B	2.68 B	2.19 B	***	NS	0.24
Rhamnus cathartica	1.25 A	1.03 B	1.08 A	1.94 A	2.69 A	4.33 A	5.84 A	***	**	0.59

Relative growth rate (g/g/d)										
Frangula caroliniana	0.05 B	0.05 A	0.05 A	0.05 A	0.04 A	0.04 A	0.03 A	**	NS	0.06
Rhamnus cathartica	0.10 A	0.06 A	0.05 A	0.04 A	0.04 A	0.04 A	0.03 A	***	**	0.43
Net assimilation rate ($g/m^2/d$)										
Frangula caroliniana	4.64 B	4.39 A	4.67 A	5.38 A	5.67 A	6.28 B	5.55 B	**	NS	0.07
Rhamnus cathartica	8.99 A	5.28 A	5.18 A	5.87 A	7.11 A	8.17 A	7.83 A	NS	**	0.17
Leaf area ratio (cm^2/g)										
Frangula caroliniana	83.51 A	83.91 A	74.25 A	64.21 A	52.20 A	42.65 A	40.64 A	***	NS	0.7
Rhamnus cathartica	78.19 A	79.79 A	69.04 A	49.72 B	40.71 B	27.29 B	24.35 B	***	NS	0.84
Specific leaf area (cm^2/g)										
Frangula caroliniana	153.0 A	158.6 A	139.9 B	124.7 A	107.8 A	99.4 A	95.4 B	***	NS	0.74
Rhamnus cathartica	151.9 A	168.8 A	155.3 A	124.8 A	112.4 A	104.5 A	106.6 A	***	NS	0.59
Leaf mass ratio (g/g)										
Frangula caroliniana	0.55 A	0.53 A	0.53 A	0.51 A	0.49 A	0.43 A	0.43 A	***	NS	0.30
Rhamnus cathartica	0.53 A	0.48 B	0.45 B	0.40 B	0.36 B	0.26 B	0.23 B	***	NS	0.77
Root mass ratio (g/g)										
Frangula caroliniana	0.21 A	0.23 A	0.23 A	0.26 A	0.27 B	0.35 B	0.32 B	***	NS	0.33
Rhamnus cathartica	0.20 A	0.20 A	0.19 A	0.27 A	0.34 A	0.43 A	0.44 A	***	**	0.77

Stem mass ratio (g/g)									
Frangula caroliniana	0.26 A	0.24 B	0.24 B	0.23 B	0.25 B	0.22 B	0.25 B	NS	NS
Rhamnus cathartica	0.27 A	0.32 A	0.36 A	0.33 A	0.30 A	0.31 A	0.33 A	NS	NS

[a] Species means within each column and parameter followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

[b] **,*** Significant at P ≤ 0.0o1 or 0.0001, respectively. NS indicates no differences.


Frangula caroliniana
A
Rhamnus cathartica
B
Relative growth rate (g/g/d)
Net assimilation rate (g/m²/d)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0
2
4
6
8
10
12
14

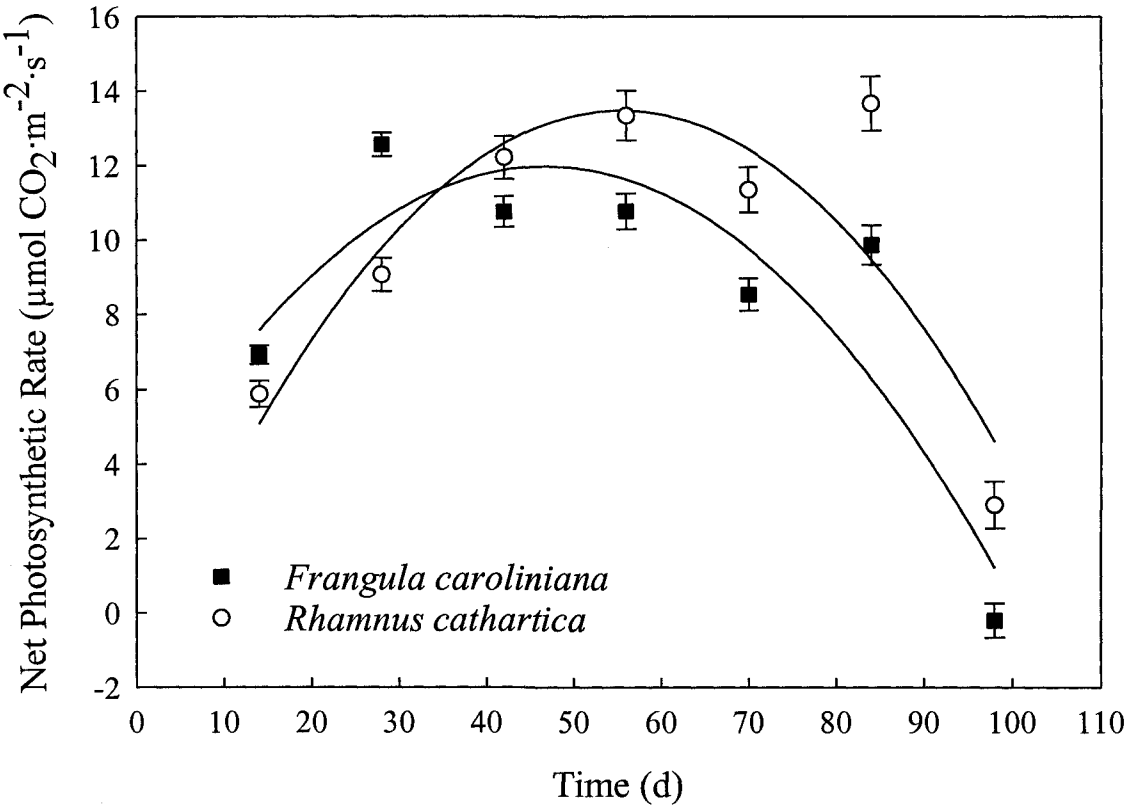


Fig. 2. Mean net photosynthetic rate of seedlings of *Frangula caroliniana* (Walt.) Gray (y = 3.001 + 0.3836[day] - 0.0041[day^2], $r^2 = 0.79$) and *Rhamnus cathartica* L. (y = - 1.5876 + 0.5433[day] - 0.0049[day^2], $r^2 = 0.77$). Values for species are means of 16 and eight replications of *F. caroliniana* and *R. cathartica*, respectively, ± SE.

CHAPTER 6. GEOGRAPHICAL PATTERN OF GENETIC VARIATION IN CAROLINA BUCKTHORN (*FRANGULA CAROLINIANA*, RHAMNACEAE) AS DETERMINED BY AMPLIFIED FRAGMENT LENGTH POLYMORPHISM ANALYSIS

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J. Ryan Stewart, William R. Graves

Key words: *Rhamnus*, population genetics, genetic variation, buckthorn

Frangula caroliniana is a shrub or small tree with a wide geographic native distribution in the southeastern United States. Populations are found as far north as Ohio, Missouri, and Indiana and as far south as western Texas and southern Florida. Despite its widespread range, little is known concerning the genetic structure of the species. Hence, we investigated genetic variation within and among 16 wild-collected populations of *F. caroliniana* by using amplified fragment length polymorphism (AFLP) markers. We detected 337 polymorphic AFLP bands after combining data from two polymerase-chain-reaction primer combinations. Ordination and clustering analyses revealed two distinct groups; the first group was comprised of plants from South Carolina, and the second consisted of the other sampled *F. caroliniana* populations from 15 states. Among populations, plants sampled from South Carolina had the highest percentage of polymorphic bands (45.4%). The range in polymorphic band percentage among the remaining populations was 0.8% (Indiana) to 31.7% (Texas). Within-population variation of the ungrouped populations was high (65.87%), which indicated high outcrossing and gene flow. Pooling

these ungrouped populations into two groups resulted in comparatively lower within-population differences (48.79%) and little variation among populations within the two groups; thus, self-fertilization may be occurring within groups. Values of the proportion of total genetic diversity (G_{ST}) revealed similar patterns of genetic structure (ungrouped G_{ST} = 0.4287; grouped G_{ST} = 0.0632). Our results, coupled with the biogeographic history of the *Frangula* genus, indicate a complex and dynamic population history of *F. caroliniana*.

Frangula caroliniana (Walt.) Gray is a shrub or small tree widely distributed in the southeastern United States (Little, 1977) along streams, on wooded rocky slopes, upland ridges, and commonly on limestone glades (Brizicky, 1964). *Frangula caroliniana* is part of Rhamnaceae, which is a cosmopolitan family that consists of nearly 50 genera and 900 species. The taxon belongs to the section *Cascara* Grub. of the *Frangula* Mill. genus, which is not universally recognized; many still consider the *Frangula* group to be a subgenus of the *Rhamnus* L. genus (Suessenguth, 1953; Brizicky, 1964; Johnston and Johnston, 1978). We accept that the elevation of *Frangula* to generic status is justified because of distinct morphological characters, which include its deciduous habit; 5-merous hermaphroditic flowers; naked winter buds; and nearly straight, pinnate leaf nervation (Grubov, 1949; Kartesz and Gandhi, 1994). In addition, the genus is distinct ecologically (Medan, 1994), and recent molecular phylogenetic data distinguish it from *Rhamnus* L. s.s. (Bolmgren and Oxelman, 2004). Despite the confusion concerning its general taxonomy, little has been done to characterize the biology of the species (Brizicky, 1964), or the structure of its population genetics.

Globally, there are up to 52 known species of *Frangula* (Grubov, 1949), with the highest concentration of species (24), located in Central and South America. Grubov (1949) recognized 24 Neotropical *Frangula* spp., which were reduced to 12 by Johnston and Johnston (1978). The large center of diversity in Central America led Wolf (1938) and McVaugh (1952) to hypothesize that *F. caroliniana* evolved from species found there.

Alternatively, Grubov (1949) concluded that *F. caroliniana* is among *Frangula* spp. that are Tertiary floral relicts that retained many features of the basal type such as large elliptic leaves with numerous straight and parallel lateral veins and simple inflorescence structures. He believed that before past continental movements, *F. caroliniana* evolved from an ancestral species that migrated across the North Atlantic land bridge into what is now eastern North America during the Eocene. Consequently, the first objective of our study was to characterize geographic structure in populations of *F. caroliniana* across its distribution range by using amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995). Our second objective was to predict the location of the origin of *F. caroliniana* by determining the geographical source of maximal genetic variation.

MATERIALS AND METHODS

Plant material—Young leaves of three to 10 individuals per population were collected from plants growing in the wild in Alabama, Florida, Georgia, Indiana, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Virginia (Table 1). Leaves were also collected from plants originally from Arkansas, Missouri, Ohio, Oklahoma, and Texas, which were grown from seeds in a greenhouse (Table 1). Approximately 25 mg of fresh leaf tissue from each source-plant was immediately snap-frozen in liquid nitrogen

and stored in a -80°C freezer until genomic DNA extraction. DNA samples were extracted using the basic CTAB extraction protocol (Doyle and Doyle, 1987).

Analyses—AFLP analysis was initiated by digesting the DNA with restriction enzymes *Eco*RI and *Mse*I; fragments were then ligated to double-stranded adapters. Reaction products were diluted 5-fold with sterile distilled, deionized H_2O. The preselective amplification step was carried out using adapter-directed primers *Eco*RI-A and *Mse*I-C. The selective amplification step was carried out on 15-fold diluted products with two fluorescently labeled primer combination sets: *Eco*RI-ACG-TET/*Mse*I-CAA and *Eco*RI-AGC-FAM/*Mse*I-CAA. All polymerase-chain reactions were performed with a thermal cycler (model PTC-0200, MJ Research, Waltham, Massachusetts). The selective amplication step was repeated twice on subsamples of each amplification product.

The reaction products of the selective amplification step were separated with an automated DNA sequencer (model ABI 377, Applied Biosystems, Foster City, California). Genescan software (Applied Biosystems) was used to interpret the AFLP fragment electropherograms. We then used Genotyper software (Applied Biosystems) to create a list based on size of fragments detected in each lane. Fragments 50 to 500 base pairs in length with peak heights >50 in the electropherograms were retained. We used Peakmatcher software (DeHaan et al., 2002) to estimate the repeatability of fragment presence or absence in each subsample. Fragments that had a repeatability of 90% were retained.

After combining the data of the two primer combination sets, we calculated Dice's (1945) coefficient of similarity between each pair of samples. Dice's coefficient was chosen because it weights band presence higher than band absence. Relationships among individuals then were estimated with ordination analysis via non-metric multidimensional

scaling, which was derived through the MDSCALE module of NTSYSpc (Rohlf, 2000). A dendrogram was then generated for data at the population level by the unweighted pair group method average (UPGMA) clustering procedure with POPGENE software (Yeh and Boyle, 1997). Goodness of fit of the UPGMA dendrogram and the similarity distance matrix was evaluated by calculating the cophenetic correlation between the two measures with the MXCOMP function of NTSYSpc.

Total genetic diversity (H_T), within-group (H_C), and within-population diversity (H_S) were calculated with POPGENE. ARLEQUIN software (Schneider et al., 2001) was used for the analysis of molecular variance (AMOVA) (Excoffier et al., 1992) at the levels of species, groups, and populations.

RESULTS

We identified 337 polymorphic bands from a total of 379 bands that were amplified from two primer combinations (Table 2). Mean repeatability of the AFLP fragments across two replications was 96.5% (Table 2). The variation in the percentage of polymorphic bands among populations ranged from 0.8% (Indiana) to 45.4% (South Carolina) (Table 3).

Ordination analysis by nonmetric multidimensional scaling revealed two groups (Fig. 1). Group 1 was comprised of plants from Alabama, Arkansas, Florida, Georgia, Indiana, Kentucky, Louisiana, Missouri, Mississippi, North Carolina, Ohio, Oklahoma, Tennessee, Texas, and Virginia. Group 2 was comprised of plants from South Carolina. There were also individual plants scattered throughout the chart representing multiple populations, but not the majority of each population (Fig. 1). The UPGMA-generated dendrogram (Fig. 2), which was in close agreement with the original distance matrix (cophenetic correlation = 0.94), confirmed the two-group structure detailed in the ordination analysis (Fig. 1). The

population from Mississippi, however, was clustered with South Carolina in the dendrogram (Fig. 2).

The species-level heterozygosity across all populations was identical to that of total genetic diversity of grouped populations (Table 4). The within-population heterozygosity of the ungrouped populations was 61% of that of the grouped populations (Table 4). At the species level, the genetic structure among all 16 populations was 0.429 (Table 4). When the populations were pooled within groups, less structure was apparent ($G_{ST} = 0.063$) (Table 4). It appears that much of the variation of the pooled populations is due to variation among the populations within groups ($G_{SC} = 0.39$) (Table 4).

At the species level, the AMOVA analysis indicated that nearly 66% of the variation in the data set was from differences within populations (Table 5). Pooling the data into groups, however, reduced the variation within populations (Table 5).

DISCUSSION

Intense levels of gene flow typically occur in fleshy-fruited species that are digested and dispersed by frugivores (Hampe et al., 2003; Petit et al., 2003), which results in low levels of among-population differentiation (Hamrick and Godt, 1996). This pattern is particularly pronounced in outcrossing woody species due to their tall stature and relatively low population densities (Hamrick and Godt, 1996), and especially in taxa with wide geographic ranges such as *Rubus* L. spp. (Petit et al., 2003), *Sorbus aucuparia* L. (Raspé et al., 2000), *Prunus* L. spp. (Mohanty et al., 2001; Mohanty et al., 2002), and *Frangula alnus* Mill. (Hampe et al., 2003). Similarly high within-population variation also was found in two Hawaiian woody species of Rhamnaceae, *Colubrina oppositifolia* Brogn. ex Mann and *Alphitonia ponderosa* Hillebr. (Kwon and Morden, 2002). Within-population variation is

typically higher in outcrossing woody species due to the decrease in genetic heterogeneity caused by long-distance pollen and seed dispersal (Mohanty et al., 2002). New alleles developing in outcrossing tree species have a high probability of being dispersed into several populations (Hamrick and Godt, 1996). Without partitioning the populations of *F. caroliniana* into groups 1 and 2 (Fig. 1), it appears that most of the genetic variation (65.87%) is found within populations (Table 5). This is consistent with the general pattern of woody species (Hamrick and Godt, 1996) and that of *F. alnus* within most of continental Europe (Hampe et al., 2003). What remains to be known, though, is whether *F. caroliniana* is a xenogamous and self-incompatible species; this would confirm our finding that outcrossing is occurring among all populations. For many years it was believed that *F. alnus* was a protandrous species (Medan, 1994), but recent work has shown that, although there is some self-incompatibility mechanism at work in *F. alnus* (Hampe, 2005), it appears not to be protandry (Medan, 1994). Hence, there is a great need to understand the reproductive biology of *F. caroliniana*, and other members of the genus. Besides the work that has been done on *F. alnus*, scant information is available.

Separating the population from South Carolina from the rest of the sampled populations presents a clearer picture of the geographic structure of the species and possibly its reproductive biology as well (Figs. 1, 2). Several genotypes, primarily from the southern portion of the range of *F. caroliniana*, were not tightly clustered within group 1 (Fig. 1). While these genotypes should not be disregarded, they did not represent the majority of their respective populations. Furthermore, there were only three genotypes sampled from the Mississippi population, and therefore the grouping of this population with the South Carolina population in the dendrogram should be interpreted with caution (Fig. 2). The variation

within populations after grouping was only 74% of within-population differences before grouping (Table 5). Cardoso et al. (2000) found that the within-population variation of wild populations of *Euterpe edulis* Mart., 57.4%, was lower than for most widely distributed and outcrossing woody species. They believed, however, that the low value was attributable to the long flowering period of the species, which increases the possibility of geitonogamy. Hence, inbreeding may partly explain the low within-population variation of not only *E. edulis*, but also in *F. caroliniana*. Although the reproductive biology of *F. caroliniana* remains uncertain, we have observed that self-fertilization can occur, at least with plants from Oklahoma (Stewart and Graves, 2004). There also appears to be little variation among populations within groups (17.24%), which also may indicate a substantial degree of inbreeding as does the difference in G_{ST} values between the species level and the grouped level; the proportion of total genetic diversity among populations within groups was only 15% of that among populations (Table 4). In contrast, the overall proportion of genetic diversity of *F. alnus* across its native range was very high ($G_{ST} = 0.81$), indicating that *F. alnus* populations in different regions of Europe have experienced distinct evolutionary processes (Hampe et al., 2003).

What caused the higher degree of genetic variation in plants from South Carolina (Table 2) when compared to the other populations? While resolution of this question is beyond the scope of this study, our results may provide hints as to the origin of the species. Rhamnaceae may be 94-96 million years old (Basinger and Dilcher, 1984; Richardson et al., 2000) or 62 million years old (Wikström et al., 2001). Although the estimated age of Rhamnaceae varies, paleobotanical data suggest that climates in the Northern Hemisphere were warm during the early Tertiary and that migrations of vascular plants, including tropical

to warm temperate species, took place between North America and Eurasia (Potts and Behrensmeyer, 1992; Qian, 2001). Grubov (1949) believed that as the climate during the Tertiary became progressively cooler and drier (Qian, 2001), the basal leaf type of large, naked, and elliptic mesomorphic leaves evolved to a varying continuum of small, hairy, and stiff xeromorphic leaf types. Grubov (1949) did not believe evolution from xeromorphic to mesomorphic features was very likely. Hence, as Bolmgren and Oxelman (2004) suggested, the modern center of diversity of Frangula in Central and South America appears to be a secondary area of diversification.

Grubov (1949) considered *F. caroliniana*, *Frangula grandifolia* (F. et M.) Grub. (Transcaucasus), *Frangula purshiana* (DC.) Cooper (northwestern United States), and *Frangula crenata* Siebold & Zucc.) Miq. (northeast Asia) to be descendants of a basal type that purportedly had a wide distribution across North America. Many of the *Frangula* spp. in the *Cascara* section, however, are native to North America and East Asia. Raven and Axelrod (1974) believed that Rhamnaceae are so well represented in tropical and temperate areas that it is difficult to trace the phylogeography of the family. Also, fossils of *Rhamnus* have been found in Alaska, and the genus is considered an element of the ancient boreotropical flora on the Bering land bridge (Qian, 2002). Further work needs to be done to clarify relationships before any conclusions can be made on the biogeographical history of the *Frangula* genus and Rhamnaceae in general (Richardson et al., 2000). Additionally, phylogenetic relationships between members of the *Cascara* section need to be clarified, which may provide further information regarding the origin and spread of *F. caroliniana*. Our work, though, is the first to show species-level genetic structure of a North American

species of *Frangula*, which helps further the understanding of this widely distributed and enigmatic genus.

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Table 1. *Frangula caroliniana* population locations and respective sample sizes.

Population location	Latitude (N)	Longitude (W)	n
Lee County, Alabama	32°36'35"	85°28'51"	10
Pope County, Arkansas	35°31'25"	92°56'38"	8
Alachua County, Florida	29°35'49"	82°25'22"	10
Walker County, Georgia	34°42'17"	85°16'55"	8
Clark County, Indiana	38°27'11"	85°40'13"	10
Hart County, Kentucky	37°14'27"	85°53'39"	9
Vernon Parrish, Louisiana	31°05'18"	93°13'39"	10
Cole County, Missouri	38°26'44"	92°18'09"	10
Oktibbeha County, Mississippi	33°27'17"	88°47'19"	3
Orange County, North Carolina	35°59'07"	79°05'41"	10
Adams County, Ohio	38°40'25"	83°27'10"	10
Johnston County, Oklahoma	34°19'40"	96°42'20"	6
Fairfield County, South Carolina	34°16'43"	81°17' 42"	7
Loudon County, Tennessee	35°43'13"	84°13'25"	10
Kerr County, Texas	30°02'50"	99°08'24"	10
Russell County, Virginia	36°57'48"	82°02'46"	10

Table 2. Amplified fragment length polymorphism (AFLP) markers obtained from two primer combinations.

Primer pair	Number of bands		Percentage polymorphic	Percentage repeatable
	Total	Polymorphic		
*Eco*RI-ACG-TET/*Mse*I-CAA	199	172	86.43	96.0
*Eco*RI-AGC-FAM/*Mse*I-CAA	180	165	91.67	97.0
Both pairs	379	337	88.92	96.5

Table 3. Polymorphic bands in each sampled population of *Frangula caroliniana*. Total number of bands for all populations was 379.

Population	Polymorphic	Percentage polymorphic
Alabama	34	9.0
Arkansas	22	5.8
Florida	61	16.1
Georgia	43	11.4
Indiana	3	0.8
Kentucky	93	24.5
Louisiana	77	20.3
Missouri	74	19.5
Mississippi	93	24.5
North Carolina	39	10.3
Ohio	15	4.0
Oklahoma	96	25.3
South Carolina	172	45.4
Tennessee	66	17.4
Texas	120	31.7
Virginia	58	15.3

Table 4. Species-level (H_T), within-group (H_C), within population (H_S) heterozygosity values, and proportion of total genetic diversity residing among populations (G_{ST}) and among groups (G_{SC}) of *Frangula caroliniana*.

Grouping	H_T	H_C	H_S	G_{ST}	G_{SC}
All populations	0.082 ± 0.011		0.047 ± 0.003	0.429	
Grouped populations	0.082 ± 0.011	0.047 ± 0.003	0.077 ± 0.01	0.063	0.39

Notes: Average ± standard deviation across all bands.

Table 5. Analysis of molecular variance (AMOVA) of 337 polymorphic amplified fragment length polymorphism (AFLP) bands from 16 populations of *Frangula caroliniana*.

Source of variation	df	Φ-statistic	Variance component	% total
All populations				
Among populations	15	$\Phi_{ST} = 0.341$	4.56	34.13
Within populations	125		8.81	65.87
South Carolina and remainder				
Among groups	1	$\Phi_{CT} = 0.340$	4.02	33.96
Among populations within groups	14	$\Phi_{SC} = 0.261$	2.04	17.24
Within populations	125	$\Phi_{ST} = 0.512$	5.77	48.79

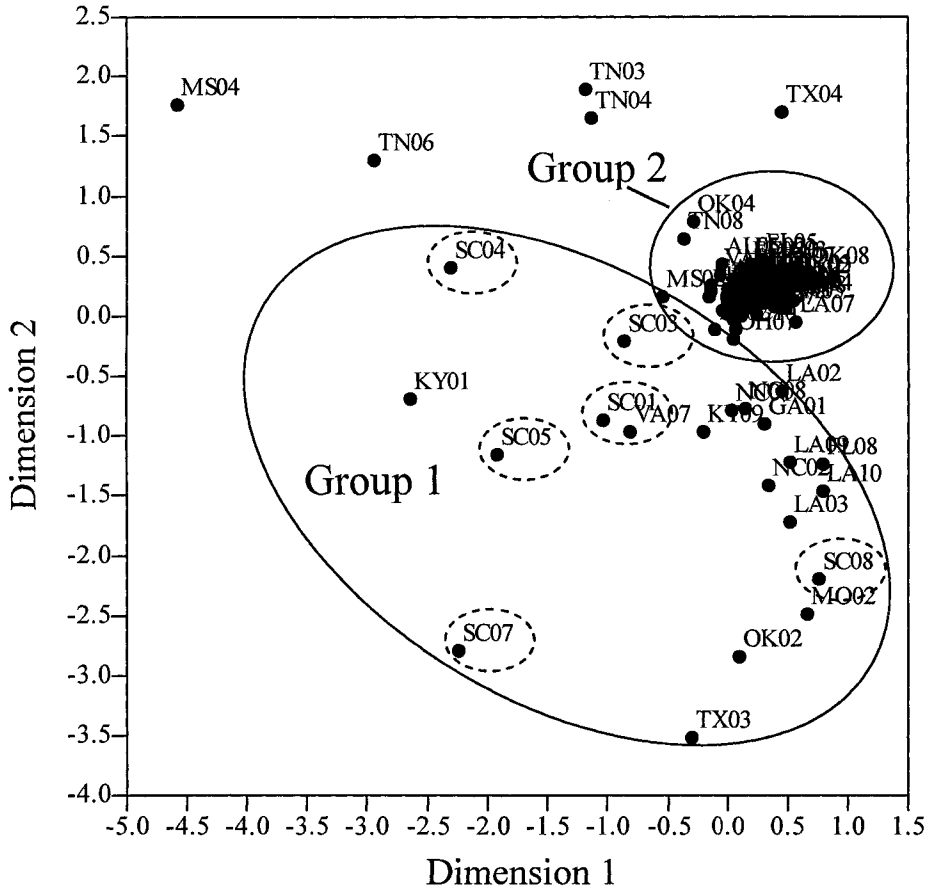
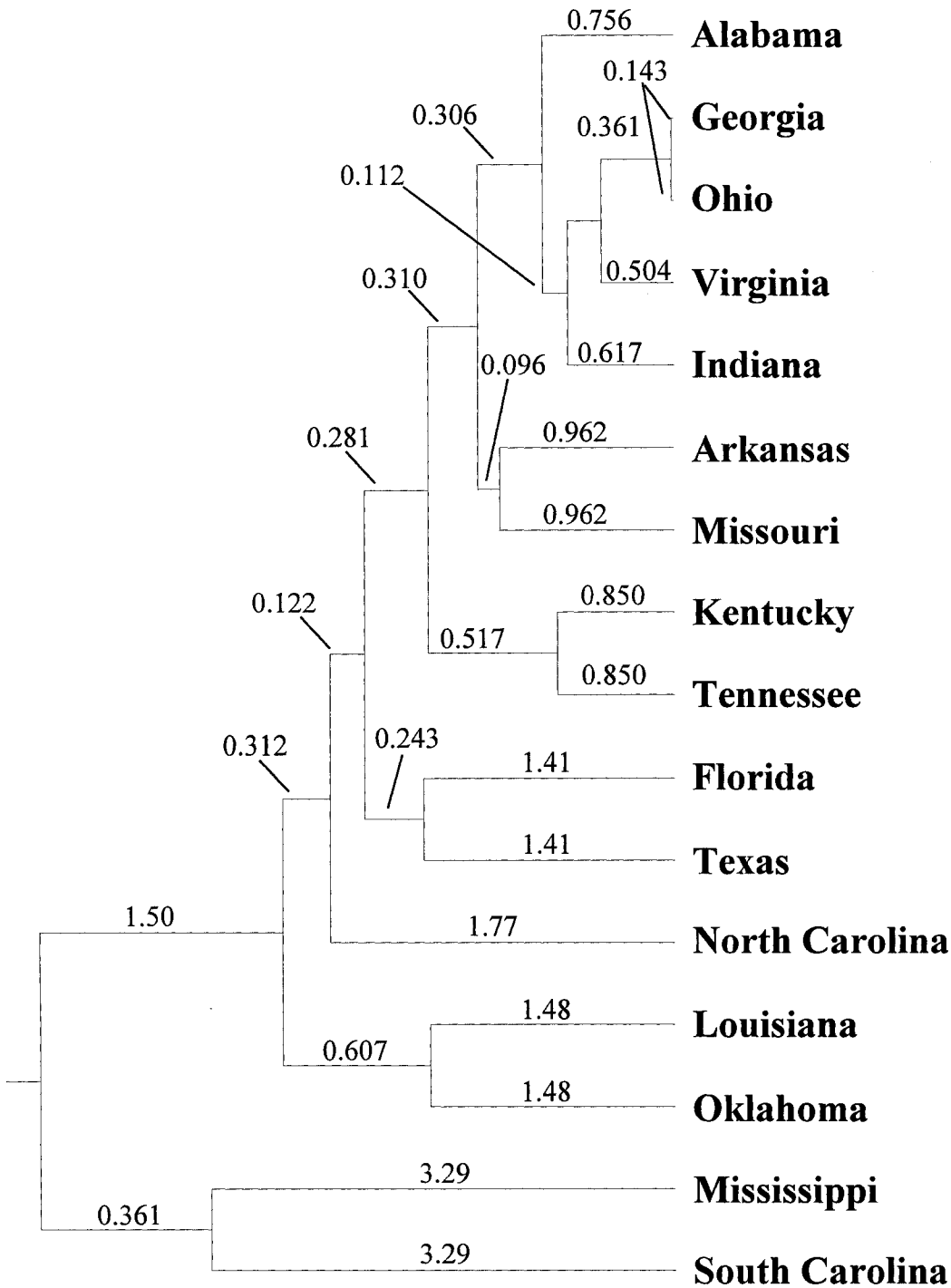


Fig. 1. Genotypes of *Frangula caroliniana* plotted from non-metric multidimensional scaling analysis of 337 polymorphic amplified fragment length polymorphisms (AFLP) bands. Each genotype is labeled with an abbreviation of its state of origin and is numbered according to the number of samples that were collected. Groups 1 and 2 are delineated by circles. Dashed lines encircle each genotype from South Carolina within group 1.


0.756
Alabama
0.143
0.306
0.361
Georgia
0.112
Ohio
0.504
0.310
Virginia
0.617
0.096
Indiana
0.281
0.962
Arkansas
0.962
Missouri
0.122
0.850
Kentucky
0.517
0.850
Tennessee
0.312
0.243
1.41
Florida
1.41
Texas
1.50
1.77
North Carolina
1.48
Louisiana
0.607
1.48
Oklahoma
3.29
Mississippi
0.361
3.29
South Carolina

CHAPTER 7. GENERAL CONCLUSIONS

Over the past several hundred years, horticulturists and plant explorers have enabled many people throughout the world to enjoy the beauty and wonder of several introduced plant taxa that otherwise may have been accessible to or seen by only a relative few. While most of these introductions are merely exotic and not invasive, several species have naturalized and have become pests of natural areas (Reichard and White, 2001). For instance, Reichard (1997) found that 82% of 235 naturalized woody species in the United States had been used in horticultural settings. Globalization has hastened and increased the introduction process, which leads to new and more complex challenges.

Hence, the general theme for this dissertation centers on the dichotomy between invasive and non-invasive woody plant species. Several traits that make a woody species horticulturally desirable also make it potentially invasive (Baker, 1974). Some of these traits include lack of special requirements for germination, high overall seed output, rapid growth from seedling to sexual maturity, and high tolerance to environmental heterogeneity and stress (Baker, 1974; Newsome and Noble, 1986; Sakai et al., 2001). Predicting invasiveness remains challenging (Mack, 1996), but can be done to improve the current understanding of the biology and ecophysiology of species that have horticultural promise, but unknown ecological risks. Typically, when a person hears the terms "*Rhamnus*," "buckthorn," or even "*Frangula*," and "introduction" in the same sentence, eyebrows are raised and questions are asked concerning the feasibility and worth of such an endeavor. As mentioned several times throughout my dissertation, the poor reputation of two exotic species (i.e., *Rhamnus cathartica* and *Frangula alnus*) has stained the good name of several North American *Rhamnus* s.l. species, including *Frangula caroliniana*. Based on the results of my

dissertation, *F. caroliniana* should be considered for further evaluation by the nursery and landscape industries.

First, I have statistically confirmed that the various edaphic conditions that *F. caroliniana* is associated with in its native range were effective predictors of its tolerance of dry to moderately wet, but not completely inundated conditions. In my travels to several native populations of *F. caroliniana*, I have seen it grow on dry, limestone glades and along rivers. Several colleagues who assisted me in my work throughout its native range have found it in similar settings. Not one of us, though, has seen the taxon growing in standing or even flowing water.

Second, one of the main causes of the aggressive spread of both *R. cathartica* and *F. alnus* is their high fecundity. My results indicate that the overall reproductive output of *F. caroliniana* is not as great as that of *R. cathartica*. Many consider the high fecundity of *R. cathartica* to be its primary means of dispersal and spread by frugivores. I also found that while cold stratification improves the seed germination of *F. caroliniana*, it has a higher degree of resistance to germination than does *R. cathartica*. Beyond the need for moist conditions, and unlike many temperate species, seeds of *R. cathartica* appear to lack any pre-germination requirements.

Third, vernal bud break of *F. caroliniana* occurs later than that of *R. cathartica*. Because early vernal budbreak has been linked to invasiveness by previous researchers, my results suggest that introduced plants of *F. caroliniana* will be less aggressive in managed landscapes than *R. cathartica* has been.

Fourth, *F. caroliniana* plants from north-central Missouri, southern Ohio, and southwestern Texas can survive colder winters than those associated with their native

habitats. There is a significant differential response, however, among these populations. Although future work needs to be done to determine the physiological northern limit of selections of this species, my work is the first to show that the geographic limit beyond which *F. caroliniana* can be planted varies with seed source.

Fifth, my growth analysis study revealed that under well-watered and nutrient-rich conditions, seedlings of *F. caroliniana* and *R. cathartica* attain similar degrees of overall fitness (i.e., total plant dry weight), but they exhibit different patterns of carbon allocation and growth to reach similar outcomes. Although high specific leaf area contributes to the high relative growth rate of many invasive species (Lambers et al., 1998; Allison and Vitousek, 2004), I found no relationship between specific leaf area and relative growth rate in *F. caroliniana* and *R. cathartica*. Instead, net assimilation rate, which has been considered a trait of successful invaders (Pattison et al., 1998), strongly correlated with relative growth rate in both species.

Sixth, my work on the population genetics of *F. caroliniana* reveals that sampled plants in South Carolina have greater genetic variation than other plants sampled across the native range of the species. This information is useful for future selection work of the species, but its application may be limited if there is uniformly poor cold hardiness of plants from South Carolina. At the phylogeographic level, my results hint at the possible origin of the species, which Grubov (1949) considered it to be derived from a basal type that had origins in what is now Eurasia.

Although I cannot state conclusively that *F. caroliniana* will not become a successful invader due to the numerous factors that contribute to invasiveness (Mack, 1996; Sakai et al., 2001), my work has shown that *F. caroliniana* has horticultural potential, and, when

compared with *R. cathartica*, the risk of introducing it into managed landscapes appears to be low.

Recommendations for Future Research

Beyond my work and the work of Bolmgren and Oxelman (2004), there is very little information, except for the conjectures of Grubov (1949), regarding the origin of the *Frangula* genus and the relationships between the many species found throughout the temperate and neotropical regions of the world. Not only is an understanding of the molecular systematics of *Frangula* important for the sake of understanding its phylogenetic relationships, but also to provide context and solutions to recent pathological problems. Recent work has revealed that at least two *Frangula* species, *Frangula purshiana* and *Frangula californica*, are associated with *Phytophthora ramorum* Werres, De Cock & Man in't Veld (sudden oak death) in California (COMTF, 2005). Further work needs to be done to understand which *Frangula* species can serve as alternate hosts to this pathogen.

It is well known that closely related members of *Rhamnus* s.s. can hybridize (Gil-Ad and Reznicek, 1997), but it is not known with certainty if interspecific hybridization can occur among members of the *Frangula* genus. Connie Parks, a former graduate student at the University of Massachusetts-Amherst, crossed two species, *F. purshiana* (egg parent) and *F. alnus* (seed parent). Although offspring did germinate, it is still unknown whether they are true offspring of both parents. Regardless, the possibility that they are raises concerns about the potential hybrid vigor that might occur and how it might affect the future spread of *F. alnus*.

Additionally, although *F. caroliniana* appears to be a generalist in terms of preferred soil pH conditions (Simpson, 1999), I believe future work is needed to determine the actual

soil pH range in which *F. caroliniana* can grow. *Frangula alnus* can grow in moist, acidic conditions. Should *F. caroliniana* prove to be invasive, it would be useful to know if it is capable of growing in similar conditions.

Several ecological comparison studies between invasive and native species have shown that, under limited-resource conditions, native plants typically outperform their invasive counterparts (Daehler, 2003). Further work needs to be done to compare *F. caroliniana* with taxonomically and/or ecologically similar invasive taxa under favorable and stressful conditions, particularly in the field (Mack, 1996).

Lastly, Rejmánek (1996) suggested that understanding the bird-dispersal preferences between native and invasive fleshy-fruited plants would improve prediction of invasive success. It would be useful to document the affinity of various bird species for fruits of *F. caroliniana*.

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APPENDIX A. MORPHOMETRICS OF *FRANGULA CAROLINIANA*

To produce a strong and consistent estimate of the genetic structure of Frangula caroliniana, I plan to couple and analyze simultaneously the molecular marker data of chapter 6 with measured morphometric characters of seedlings of the taxon from multiple native seed sources. Seeds were collected in the summer of 2004 from 10 plants in Lee County, Alabama (lat. 32°36'35"N; long. 85°28'51"W), from two plants in Van Buren County, Arkansas (lat. 35°37'01"N; long. 92°16'03"W), from two plants in Gwinnett County, Georgia (lat. 33°59'17"N; long. 84°10'49"W), from five plants in an unknown location (i.e., I cannot find the location information at the present time) in Georgia, from nine plants in Vernon Parrish, Louisiana (lat. 31°05'18"N; long. 93°13'39"W), from 10 plants in Cole County, Missouri (lat. 38°26'44"N; long. 92°18'09"W), from five plants in Orange County, North Carolina (lat. 35°59'07"N; long. 79°05'41"W), from five plants in Adams County, Ohio (lat. 38°40'25"N; long. 83°27'10"W), from 10 plants in Johnston County, Oklahoma (lat. 34°19'40"N; long. 96°42'20"W), from nine plants in Loudon County, Tennessee (lat. 35°43'13"N; long. 84°13'25"W), and from nine plants in Kerr County, Texas (lat. 30°02'50"N; long. 99°08'24"W). All seeds were stratified for 108 d at 4 °C and germinated in a greenhouse.

Seedlings were planted in 10.2-cm-diameter pots (height = 10.2 cm; volume = 459 cm^3) and were grown under greenhouse conditions with a daily 16-h photoperiod. Two 400-W, high-pressure sodium lamps were used to supplement natural irradiance. The plants were irrigated every 2-3 d and were fertilized every 6-9 d with a solution of 11.0-mM N from a mixture of Peters Excel All-Purpose and Cal-Mag (16.5N-2.2P-13.5K) (Scotts, Marysville, Ohio) in tap water.

Morphometric characters were measured on the newest fully expanded leaf of each plant on 24 and 25 June 2005. Measured characters included leaf length, leaf width at widest axis, leaf length:width ratio, distance from proximal end of leaf blade to its widest axis, number of secondary leaf veins on one side of midrib, veins per cm of leaf length, leaf shape (distance from proximal end of leaf blade to its widest axis/leaf length), leaf apex angle (full angle, both sides of midrib), vein angle (between midrib and second secondary vein on one side of midrib), and stem length (length of the primary stem from the cotyledonary node to the apex) (Table 1).

Table 1. Mean morphometric values of 119-day-old seedlings of *Frangula caroliniana* from multiple native seed sources. Standard errors are below each mean in parentheses.

Character and sample size	Population									
	Alabama	Arkansas	Georgia	Louisiana	Missouri	North Carolina	Ohio	Oklahoma	Tennessee	Texas
Leaf										
Length (cm)	9.41	8.35	10.5	8.68	6.98	10.69	7.81	9.93	6.43	7.37
	(0.23)	(0.58)	(0.4)	(0.24)	(0.23)	(0.25)	(1.06)	(0.24)	(0.25)	(0.26)
Width (cm)	2.78	2.52	3.02	2.45	2.39	3.43	2.34	2.98	2.05	2.94
	(0.07)	(0.13)	(0.12)	(0.06)	(0.05)	(0.1)	(0.28)	(0.08)	(0.06)	(0.1)
Length:width ratio	3.42	3.33	3.5	3.58	2.92	3.14	3.33	3.38	3.17	2.53
	(0.06)	(0.2)	(0.09)	(0.09)	(0.08)	(0.06)	(0.27)	(0.07)	(0.12)	(0.07)
Distance to widest axis (cm)	4.75	3.53	4.62	3.95	3.63	4.81	3.71	4.23	3.02	3.56
	(0.2)	(0.4)	(0.24)	(0.19)	(0.16)	(0.24)	(0.56)	(0.19)	(0.12)	(0.17)
Number of secondary veins	7.64	7.5	8.27	7.76	6.74	9.32	7	8.14	6.45	7.51
	(0.2)	(0.56)	(0.28)	(0.21)	(0.2)	(0.27)	(0.84)	(0.2)	(0.21)	(0.23)
Secondary veins per cm	0.82	0.92	0.81	0.91	0.99	0.88	0.91	0.83	1.04	1.05
	(0.02)	(0.08)	(0.03)	(0.03)	(0.03)	(0.02)	(0.05)	(0.02)	(0.04)	(0.03)
Shape (dist. to widest axis/length)	0.5	0.43	0.44	0.45	0.52	0.45	0.47	0.42	0.48	0.49
	(0.02)	(0.04)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.02)

Apex angle (°)	61.02	56.1	56.73	60.16	64.2	68.2	63	60.9	60.9	80.98
	(1.51)	(5.49)	(1.54)	(1.64)	(1.28)	(1.74)	(2.95)	(1.16)	(2)	(2.31)
Vein angle (°)	37.3	36.5	37.42	36.24	34.06	42.68	31.6	36.06	33.03	41.56
	(2.74)	(1.42)	(1.02)	(1.16)	(0.55)	(0.89)	(1.86)	(2.48)	(0.64)	(2.49)
Stem height (cm)	11.06	7.67	13.34	10.38	5.4	14.83	5.76	14.17	5.36	10.14
	(0.53)	(0.43)	(0.9)	(0.58)	(0.2)	(0.76)	(0.33)	(0.57)	(0.31)	(0.66)
n	50	10	33	38	50	25	5	50	40	43

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